Molecular typing – Part 1

**P509** One platform and multiple assay formats: mass spectrometry for molecular typing of the *Mycobacterium tuberculosis* complex

C. Honisch*, S. Niemann, M. Moink, C. Arnold, S. Gharbia (San Diego, US; Borstel, DE; London, UK)

**Objectives:** The analysis of nucleic acids by mass spectrometry (MS) has evolved to a user-friendly technology for characterising DNA, and RNA in clinical research and molecular medicine. Recently, the technology has become a versatile tool for microbial identification utilising comparative sequence analysis as shown for 16S based typing of mycobacteria [1]. Here, we present examples for the development of MS specific assays for molecular typing of the *M. tuberculosis* Complex including spoligotyping and antibiotic resistance identification, which is essential for epidemiological analysis. One technology, the MassARRAY® platform can be used with different assay formats and typing schemes to obtain results from species to strain identification in an automated fashion.

**Methods:** Nucleic acid analysis by MS is based on PCR amplifications using unique primer sets.

Traditional spoligotyping detects the presence or absence of 43 different spacer sequences. For MS 43 spacer oligonucleotide probes were designed and the presence of a spacer is detected by base extension (TypePLEX®).

Resistance regions are amplified by PCR with a tagged primer system in multiplex followed by in vitro transcription of both DNA strands. Subsequent endonuclease digests of the RNA transcripts at the bases cytosine and uracil result in four mixtures of RNA cleavage products were designed and the presence of a spacer is detected by base extension (TypePLEX®). Resistance is identified by correlating acquired spectra with theoretical peak patterns predicted for in silico cleavages of sequences contained in a reference database. Microheterogeneities are identified and deliver new resistance types.

**Results:** Over 200 characterised strains from different reference centres representing the major *M. tuberculosis* Complex lineages were run over the established spoligotyping and resistance assays. Results were in concordance with traditional spoligotyping and dideoxy sequencing data. Advantages of the MS approach are the homogeneous assay formats without any clean-up steps, semi-automated processing, the time-to-result with a throughput of 192 samples in 8 hours for spoligotyping and plexing capabilities for comparative sequence analysis of multiple genomic regions in one reaction.

**Conclusion:** Mass spectrometry specific assay formats for genotyping and comparative sequence analysis generate highly accurate qualitative and quantitative data and provide a toolbox for molecular typing of microbes and viruses.

**Reference(s)**


**P510** Application of MALDI-TOF mass spectrometry for *Helicobacter pylori* study

E. Ilina*, V. Vereshchagin, M. Serebrayakova, V. Chelysheva, A. Boroszkaya, K. Momynaliev, T. Maier, M. Kostrzewa, V. Goorun (Moscow, RU; Bremen, DE)

**Objectives:** The applicability of MALDI TOF mass spectrometry techniques for investigation of a highly variable bacterium such as *Helicobacter pylori* was studied.

**Methods:** *H. pylori* were grown on Columbia agar plates (BioMerieux, France) at 37°C and 5% CO2 for 48 hours. Fresh bacterial colonies were transferred into 300 μL of water. After precipitation with ethanol (900 μL) and centrifugation the pellet was suspended in 20 μL of 50% acetonitrile, 35% formic acid, and analyzed in a microflexTM (Bruker Daltonics, Germany) using a saturated solution of α-CHCA as matrix. Spectra analysis and species identification was done using flexAnalysis 2.4 and MALDI Biotyper 2.0 software (Bruker Daltonics, Germany). Mass spectra of protein fragmentation were obtained by an Ultraflex II MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany) equipped with Smart BeamTM laser.

**Results:** 17 clinical strains as well as two laboratory strains of *H. pylori* were analyzed by MALDI TOF MS. Mass spectra collected were found containing 7–13 significant peaks per sample, and only five protein signals were identical for more than 70% of strains. Four of them were matched to ribosomal proteins. The fifth reproducible peak with m/z 6948 was assigned to histidine-rich metal binding polypeptide by MS/MS. In spite of evident intra species protein heterogeneity of *H. pylori* the mass spectra collected for a particular strain under the several cultivations were reproducible. Moreover, all clinical strains were perfectly identified as *H. pylori* by MALDI Biotyper 2.0 software using a database containing mass spectra from different bacterial strains (n = 3290) including *H. pylori* 26695 and J99.

**Conclusion:** MALDI TOF MS fingerprinting is a suitable tool for *H. pylori* species identification and typing and could help in better understanding of transmission pathways of this bacterium.

**P511** *Helicobacter pylori* genotypes in different ethnic groups resident in Tehran, Iran


**Objectives:** There is a geographic variation in *Helicobacter pylori* genotypes. cagA and cagE, opA and vacA genotypes of *H. pylori* are associated with peptic ulcer disease (PUD). This study compared the distribution of these genotypes in major ethnic groups residing in Tehran, Iran and their association with clinical outcomes.

**Methods:** *H. pylori* infected patients proven by culture were recruited prospectively. DNA was extracted from isolated *H. pylori* and PCR was carried out to determine the cagA, cagE and opA status and vacA alleles.

**Results:** A total of 124 patients living in Tehran were enrolled in this study. The ethnic distribution was 74 Persian, 33 Turkish and other ethnicities including 7 Kurdish, 5 Lurs, 3 Afghani and 2 Arab patients. The predominant vacA signal region genotype was s1 among isolates from all ethnicities. The vacA middle region genotype m2 was predominant in Persian and Turks. Of the Persian, Turkish and other ethnic isolates, 64.9%, 72.7% and 70.5%, respectively, were cagA positive, and 47%, 30% and 76.5%, respectively, were cagE positive. The opA gene was present in 51.4% of Persian, 33.3% of Turks and 70.5% of others ethnic isolates.

**Conclusion:** There is difference in the *H. pylori* strains among the ethnic groups in Iran. However, there was no significant association between cagA, cagE and opA status or vacA genotypes and clinical outcomes in Iranian patients irrespective of ethnic groups. None of these markers were helpful in predicting the clinical presentation of a *H. pylori* infection in Iran.
The number of *Helicobacter pylori* CagA EPIYA C tyrosine phosphorylation motifs is associated with histological features of chronic gastritis

R.M. Ferreira*, J.C. Machado, F. Carneiro, C. Figueiredo (Porto, PT)

Background and Aims: *H. pylori* strains containing CagA are more virulent than CagA-negative strains and are associated with increased gastric carcinoma risk. CagA may undergo phosphorylation on tyrosine residues within Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs, present in the C-terminus. In Western strains EPIYA motifs are classified as EPIYA-A, -B, and -C. The number of EPIYA C motifs influence the level of CagA tyrosine phosphorylation, the degree of SHP-2 binding, and the magnitude of cytoskeleton changes induced by *H. pylori*. The aim of this study was to characterise *H. pylori* CagA EPIYA motifs in strains infecting Portuguese patients in order to explore their relationship with the histopathological features of chronic gastritis.

Materials and Methods: 117 *H. pylori*-infected patients with chronic gastritis were studied. *H. pylori* density, chronic inflammation, polymorphonuclear activity, epithelial damage, glandular atrophy, and intestinal metaplasia were assessed according to the Sydney system. One biopsy specimen from the antrum was used for DNA isolation and genotyping. The presence of cagA was determined by PCR using primers flanking the EPIYA-coding regions. cagA-positive cases were genotyped for the presence of 1, 2, or 3 EPIYA C motifs: the odds ratio for atrophy was 5.4 (95% CI, 1.6−18) higher grade of polymorphonuclear activity, epithelial damage, glandular atrophy, and intestinal metaplasia (P=0.0006 and P=0.002, respectively). No associations were observed between i region genotypes and *H. pylori* colonisation density. vacA s1, m1, and i1 strains were strongly associated with gastric carcinoma (all P <0.0001).

Conclusions: *H. pylori* vacA i1 strains are associated with more severe histological features of chronic gastritis and increased gastric carcinoma risk in the Portuguese population.

Evaluation of use of multiple locus variable number tandem repeat analysis for typing of *Pseudomonas aeruginosa*

J. Turton*, S. Turton, S. Yardie, M. Kaufmann, T. Pitt (London, UK)

Objectives: To establish and evaluate a method using Variable Number Tandem Repeat (VNTR) loci suitable for routine typing of *Pseudomonas aeruginosa* in a reference laboratory.

Methods: PCR amplification of up to 12 VNTR loci was carried out on a panel of isolates previously characterised by pulsed-field gel electrophoresis (PFGE) and on serotype reference strains. Repeat numbers at targets with small repeat units were determined using fluorescent forward primers and sizing on a sequencer. Repeat numbers at the remaining loci were determined by agarose gel electrophoresis. A scheme using eight loci was adopted and tested on a further 100 isolates, also typed by PFGE.

Results: The twelve loci initially used were reduced to eight, with no loss of discrimination, at least among the 40 isolates originally tested. Of the 28 PFGE types represented by the 77 isolates in the panel, 27 were successfully distinguished by their repeat numbers. The method successfully distinguished all 17 serotype reference strains, none of the profiles of which matched one another, or any of the isolates within the panel or routine isolates tested. Repeat numbers at the eighth locus could provide discrimination within a PFGE type. Representatives from outbreaks, received within a short space of time, all shared the same number of repeats at this locus. In contrast, isolates of the Liverpool strain from CF patients showed variation in repeat number at this locus, even among isolates from the same centre. Agreement with PFGE was good for the further 100 isolates tested, but in two instances, the VNTR analysis failed to distinguish pairs of isolates that were distinct by PFGE, except at locus 61.

Conclusion: In the vast majority of cases, VNTR analysis at these eight loci provided discrimination at a level similar to that afforded by PFGE, with most strains being identified unambiguously. Results could be obtained within a day, leading to significant improvements in reporting times.
Comparison of opr sequences for the characterisation of clinical isolates of Pseudomonas aeruginosa


Objectives: Allelic variation has been described in the genes encoding the outer membrane proteins of P. aeruginosa (Pirmay et al. 2002). oprD, oprI, and oprL show potential for use in the epidemiological typing of strains. We determined the partial oprI, oprL and oprD sequences on a set of epidemiologically diverse clinical isolates of P. aeruginosa, some from cystic fibrosis (CF) patients that had previously been characterised by pulsed-field gel electrophoresis (PFGE). We also explored associations of oprD sequence variation and predicted structure with imipenem susceptibility of the isolates.

Methods: oprI and oprL and partial oprD (corresponding to nt 701–1269 of the coding sequence of PA01) sequences of 30 isolates of P. aeruginosa representing 26 distinct PFGE profile types were determined and aligned. Clustering of each gene was displayed in an UPGMA dendrogram. The MICs of imipenem for the isolates was determined by agar dilution.

Results: Both oprI and oprL sequences showed little variation and did not define clear groupings within the panel. For oprD sequences the isolates fell into 2 clear sequence type groups, one of which was further divided into 2 subgroups (1A and 1B). Isolates with the same PFGE type invariably belonged to the same oprD sequence type group. Ten of the 12 isolates from CF patients, including those belonging to lineages affecting multiple centres, fell in subgroup 1A. Each group included some representatives with various sequence disruptions, such as stop codons (3 isolates), frameshift mutations (2), deletions (2) and an insertion sequence (ISPa20). Those with these disruptions exhibited imipenem resistance (MIC ≥ 16 mg/L).

Conclusion: OprD sequences of P. aeruginosa fall into 2 distinct phylogenetic groups with those from CF patients mainly clustering into a single group. Disruptions in the sequence were common and sometimes extreme and were associated with imipenem resistance. Sequence differences between the two groups can be exploited for strain characterisation and may provide insights into the relationships between strains.

The highest numbers of cases in category 2 were found in 1998 and 2003, 2 years for which the hypothesis of patient-to-patient transmission within this category was supported by plausible epidemiological links in 61% and 85%, respectively, of the cases who shared a common genotype, and by lower figures after enforcement of infection control practice in the following years. In the 3 other study years, a plausible epidemiological link was only found in 23 to 25% of cases. The number of cases in category 3 (unique genotype) remained stable over the years, suggesting that endogenous source was constant during this period of time.

Conclusion: Molecular typing of P. aeruginosa in ICU patients allows to better understand the epidemiology in this setting and to evaluate the impact of control measures. Cases of endogenous source appear to remain stable over the years.

Table: Molecular epidemiology of P. aeruginosa cases in ICUs between 1998 and 2007

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of ICU admissions</th>
<th>No. of cases</th>
<th>No. of cases/1000 admissions</th>
<th>Cat. 1</th>
<th>Cat. 2</th>
<th>Cat. 3</th>
<th>Not typed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>2367</td>
<td>142</td>
<td>60</td>
<td>6.5</td>
<td>23.7</td>
<td>15.6</td>
<td>4.2</td>
</tr>
<tr>
<td>2000</td>
<td>2446</td>
<td>74</td>
<td>60</td>
<td>7.0</td>
<td>23.7</td>
<td>13.1</td>
<td>3.7</td>
</tr>
<tr>
<td>2003</td>
<td>2400</td>
<td>85</td>
<td>30.3</td>
<td>35.4</td>
<td>0.4</td>
<td>11.3</td>
<td>7.1</td>
</tr>
<tr>
<td>2004</td>
<td>2554</td>
<td>83</td>
<td>32.5</td>
<td>32.5</td>
<td>0.4</td>
<td>11.3</td>
<td>7.1</td>
</tr>
<tr>
<td>2007</td>
<td>2675</td>
<td>87</td>
<td>32.5</td>
<td>32.5</td>
<td>0.4</td>
<td>11.3</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Molecular epidemiology of Pseudomonas aeruginosa in intensive care units over a 10-year period (1998–2007)

M. Cuttelod*, L. Senn, V. Terletsky, I. Nahimana, C. Petignat, P. Egginmann, J. Belle, A. Wenger, G. Zanetti, D.S. Blanc (Lausanne, CH)

Background: P. aeruginosa is one of the leading nosocomial pathogens in ICUs. The source of this microorganism can be either endogenous (digestive flora) or exogenous (other patients, the contaminated environment such as sinks or taps). The proportion of cases due to transmission (patient-to-patient or from the environment) in this setting is still debated, and is important for implementing appropriate control measures.

Objectives: To determine the relative importance of exogenous versus endogenous sources of P. aeruginosa in ICU patients over 10 years.

Methods: Molecular typing was performed on all P. aeruginosa isolates obtained from routine swabs of the inner part of the ICU taps. It was compared with typing of P. aeruginosa isolated in clinical specimens of ICU patients in 1998, 2000, 2003, 2004, and 2007. This allowed the division of patients with clinical specimens yielding P. aeruginosa (thereafter: cases) into 3 categories:

1. Cases with isolate identical to one found in taps
2. Cases with isolate identical to one of at least another case, but not found in taps
3. Cases with isolate of a unique genotype

Results: Results are presented in the table. The number of cases in category 1 was high in 1998 and decreased significantly thereafter, presumably as a result of enhanced control measures since 1999.

The number of cases in category 2 were found in 1998 and 2003, 2 years for which the hypothesis of patient-to-patient transmission within this category was supported by plausible epidemiological links in 61% and 85%, respectively, of the cases who shared a common genotype, and by lower figures after enforcement of infection control practice in the following years. In the 3 other study years, a plausible epidemiological link was only found in 23 to 25% of cases. The number of cases in category 3 (unique genotype) remained stable over the years, suggesting that endogenous source was constant during this period of time.

Conclusion: Discriminatory Indices between these typing methods are similar and congruence as measured by an r value and Wc is very high between all methods. MLVA and MLST are comparable typing techniques with

Assessing clonal relatedness of Pseudomonas aeruginosa isolates: MLV should be preferred over MLST and PFGE

R. van Mansfeld*, M.J.M. Bonten, R. Willem (Utrecht, NL)

Objectives: Pulsed-field gel electrophoresis (PFGE) is generally considered the gold standard for typing of Pseudomonas aeruginosa. The last decade PCR and sequence-based molecular typing techniques have been developed, like multiple-locus-variable number tandem repeat analysis (MLVA) and multi-locus-sequence typing (MLST), which are faster, easier to interpret, better reproducible among laboratories, and that generate unambiguous typing data. To investigate whether MLVA and MLST can replace PFGE for genotyping we typed 32 strains with each method and compared typing results.

Methods: 32 P. aeruginosa strains, derived from sputum samples of Dutch CF patients, were typed by PFGE, MLST and MLVA. MLST was based on the scheme designed by Curran et al. (JCM, 2004) and the MLVA typing method was an adjustment of the Vu-Thien scheme (JCM, 2007), using only 9 of the original 15 VNTR (variable number of tandem repeats) loci. The discriminating ability of these methods was determined as well as the congruence among the different techniques by using the Adjusted Rand Index (aRI) and Wallace coefficient (Wc). MLST, MLVA and PFGE types were compared. Clonal clusters were defined as isolates with PFGE banding pattern similarity of >80% or with MLST and MLVA profiles with a maximum of one locus difference.

Results: In the set of 32 isolates PFGE, MLVA, and MLST distinguished 28, 27, and 21 types, respectively and 21, 21, and 20 clonal clusters. All three methods have Discriminatory Indices (DI) with overlapping 95% confidence intervals (CI) of 98.8 (CI 97.4–100), 98.0 (CI 95.9–101) and 95.2 (CI 91.4–98.9) for PFGE, MLVA and MLST, respectively. Congruence testing between the methods, at the level of clonal clusters, showed an aRI of 0.97 for PFGE vs. MLVA and 0.94 for PFGE vs. MLST. We between PFGE and MLVA is 0.97 both ways. We between PFGE and MLST was also 0.97, while MLST had a slightly lower prediction of partition by PFGE of 92% (Wc 0.92).

Conclusion: Discriminatory Indices between these typing methods are similar and congruence as measured by an r value and Wc is very high between all methods. MLVA and MLST are comparable typing techniques with
respect to portability and ease of interpretation. This is a huge advantage compared to PFGE. In this study MLVA is slightly more congruent to PFGE than MLST. And, MLVA is cheaper as it does not require sequencing. Therefore, we suggest MLVA as first choice typing method for characterising clonal relatedness of P. aeruginosa isolates.

**P518** MLST reveals a polyclonal structure of *Pseudomonas aeruginosa* from initial and early colonisation stages in cystic fibrosis patients
A. Fernandez-Olmos*, R. del Campo, A. Lamas, P. Ruiz Garbajosa, L. Maiz, F. Baquero, R. Cantón (Madrid, ES)

Objectives: MLST typing schemes have been scarcely apply to *Pseudomonas aeruginosa* isolates from cystic fibrosis (CF) patients. The aim of this study was to assess the population structure of *P. aeruginosa* isolates from initial and colonisation stages in CF patients followed by our CF-Unit from 1994 to 2007.

Methods: Twenty-four *P. aeruginosa* isolates (20 first *P. aeruginosa* isolate in the patient, four 2nd-4th isolate), from 21 CF patients (median age 9 years, range 0–34) were typed by Spel-PFGE and MLST (Curran et al. 2004, http://pubmlst.org/paeruginosa) using the housekeeping genes acrA, aroE, gaa, mduB, nuoD, ppaA and trpE. Median follow-up period of these patients were 6.9 years (range 1–14). The eBURST algorithm (http://pubmlst.org/analysis/) was used for phylogenetic analysis.

Results: Spel-PFGE identified unique patterns (n = 21) from 19 unrelated patients with single isolates, and from the other two patients with multiple isolates (two and three, respectively, collected separately from more than two years). However, PFGE profiles with 6 bands of difference were observed in two pairs of unrelated patients. Moreover, 20 different sequence types (STs) were identified within studied isolates. Within four isolates with PFGE profiles with 6 bands of differences, two STs were identified. A group of 3 patients carried single locus variants.

Conclusions: Unlike other studies, a polyclonal structure of *P. aeruginosa* from initial and early colonisation stages in CF patients were observed with no previously identified epidemic clones. Moreover, the finding of different genotypes in the same patient during a long term follow-up period suggests ephemeral persistence of initial colonising strains.

**P519** The applicability of three different methods for the molecular typing of *Pseudomonas aeruginosa*
C. Ratkai, S. Quinteira, F. Grasso, E. Nagy*, L. Peixe (Szeged, HU; Porto, PT)

Objectives: *Pseudomonas aeruginosa* is a Gram-negative rod causing serious infections, often isolated from nosocomial outbreaks. Several methods have been developed for the typing to determine the relatedness of these nosocomial pathogens, such as serotyping, ribotyping, or PCR based methods. For epidemiologists a reliable, cheap and rapid typing method is essential for measuring the effectiveness of the infection control, and in case of the increasing number of resistant *P. aeruginosa* isolates, the ability to decide if it is due to patient-to-patient transmission.

Methods: During the period of 2004 to 2008 we isolated 25 carbapenem resistant non-mucoid *P. aeruginosa* isolates from different non-cystic fibrosis patients, hospitalised in nine different hospital wards of South-Hungary, and 18 *P. aeruginosa* isolates from cystic fibrosis patients. We compared different typing methods for these *P. aeruginosa* isolates, namely pulse-field gel-electrophoresis (PFGE), which is considered to be the “gold-standard” method for molecular typing of *P. aeruginosa*; multilocus sequence typing (MLST), which is based on the allelic differences in certain housekeeping genes; and the DiversiLab typing system (BioMérieux), which is based on repetitive element-based PCR (rep-PCR).

Results: In case of the carbapenem-resistant isolates we determined eleven different pulso-types with the PFGE, and twelve different types with the rep-PCR. Contradictory results were obtained in case of three of the 25 (12%) isolates with these two typing methods. Twenty-one of the 25 isolates were members of three different outbreaks observed in the intensive care unit, and according to the MLST analysis they belong to different clonal complexes. In case of the strains isolated from cystic fibrosis patients 10 pulso-types and 12 different types with the rep-PCR were determined. Contradictory results were obtained in case of seven of the 18 (39%) isolates with these two typing methods. According to the MLST analysis, these strains are singletons.

Conclusion: Our experiences suggest, that the PFGE had a high discriminatory power, but it is limited by the technical complexity, expense and time. Only the MLST method provides data about the clonal relationship of the isolates, but it is not applicable in case of local outbreaks. Rep-PCR is suitable as a rapid epidemiological surveillance tool, however, mainly in case of strains from cystic fibrosis patients, it proved to be too discriminating.

**P520** Molecular characterisation of *Candida parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis* clinical isolates in a tertiary-care hospital
M. de Toro, M.J. Torres, M. Ruiz, J. Aznar* (Seville, ES)

Objectives: To describe the prevalence of the recently renamed species *Candida orthopsilosis* and *Candida metapsilosis* in candidaemia and identified them in isolates recovered from different body sites.

Methods: Fifty-six clinical isolates recovered from blood cultures obtained from patients with candidaemia (study period 2003–2008), and 74 isolates from a variety of sources from some in-hospital and outpatient were studied. These isolates were previously identified as *Candida parapsilosis* by conventional laboratory tests. Molecular characterisation of the clinical isolates was performed by RAPD test using the primer RPO2 (5′-GGGATCCCCA-3′), and by Banl digestion profile analysis of a fragment of the secondary alcohol dehydrogenase gene. *C. parapsilosis* ATCC 22019, *C. orthopsilosis* ATCC 96139 and *C. metapsilosis* ATCC 96144 were also included as reference strains.

Results: Between 2003 and 2008, a total of 381 cases of candidaemia were detected in our hospital, and 95 (24.9%) of them corresponded to *C. parapsilosis*. Based on molecular criteria, 10 of the 130 studied isolates were identified as *C. orthopsilosis* (6 of them were bloodstream strains and 4 of them were from other sources), and 1 as *C. metapsilosis* (recovered from a skin sample). The remaining 119 isolates were identified as *C. parapsilosis*. Based on these results, *C. orthopsilosis* accounted for 7.7% (10/130) of all strains studied, but accounted for 10.7% (6/56) of the *C. parapsilosis* bloodstream strains and for 5.4% (4/74) of the strains from other sources. *C. metapsilosis* accounted for 0.77% (1/130).

Conclusions: The prevalence of the recently described species *C. orthopsilosis* in candidaemia is significant and its characterisation can be recommended. Further studies are needed to establish the clinical significance of *C. metapsilosis* isolates in our area.

**P521** A new method PCR MP for *Candida albicans* strains genotyping
B. Krawczyk, J. Leibner-Ciszak, A. Mielech*, A. Sledzinska, A. Samet, J. Kaur (Gdansk, PL)

Objectives: Infections with *Candida albicans* strains can be a serious medical problem. It is necessary to establish the genetic relatedness of clinical isolates. The aim of this study was the elaboration of a new PCR MP method for genetic typing of *Candida albicans* strains, designed for epidemiological studies. The principle of the method is to use low denaturation temperatures during the ligation mediated PCR (LM PCR). The optimisation of the method has been carried out using different restriction enzymes and denaturation temperature. We evaluated the typeability and discriminatory power of this technique in comparison to RAPD and REA-PFGE. We also validated the reproducibility of the PCR MP method.

Methods: A total of 123 *Candida albicans* strains (including 7 reference, 11 clinical unrelated, and 105 isolates from different geographic origins...
and patients of two hospitals in Poland) were examined. Typing of candidal strains to determine their genetic relatedness has been done by PCR MP, REA-PFGE and RAPD methods.

**Results:** The genotyping results of the PCR MP were compared with results from macrorestriction analysis of the chromosomal DNA by pulsed-field gel electrophoresis (REA-PFGE) and RAPD techniques. Digestion of the chromosomal DNA with the Smal endonuclease and separation of the fragments by PFGE revealed 26 unique types. Application of RAPD resulted in recognition of 25 types. Strains were grouped into 27 types using PCR MP.

**Conclusion:** Data presented here show for the first time the evaluation of PCR MP technique for candidal strains differentiation. The results showed that the PCR MP technique has at least the same discriminatory power as REA-PFGE and RAPD. We propose that PCR MP can be used as an alternative technique in large-scale hospital studies of intra-species genetic relatedness of *Candida albicans* strains.

**P522 Multilocus sequence typing of sequential *Candida albicans* isolates from patients with persistent or recurrent fungaemia**

D.A. da Matta, J.S.A. Melo*, T. Guimarães, M.E. Brandt, T.J. Lott, A.L. Colombo (São Paulo, BR; Atlanta, US)

Multilocus sequence typing (MLST) is a useful tool in understanding the phylogenetics and epidemiology of *Candida albicans* strains from invasive candidiasis.

**Objective:** Our goal was to determine whether indistinguishable or different strains were responsible for persistent or recurrent fungaemia by performing MLST and ABC typing, which is based on the presence or absence of an intron in the 26S rDNA region, on sequential *C. albicans* isolates from the same patient.

**Methods:** We applied MLST to 21 *C. albicans* strains from 8 patients with persistent or recurrent candidaemia collected during a multicentre surveillance study in 4 public tertiary care hospitals in Brazil. Persistent candidaemia was defined as an episode of candidaemia occurring at least 1 month after the apparent complete resolution of an infectious episode caused by the same *Candida* species.

**Results:** All the patients’ strains but one showed the same MLST diploid sequence type (DST), ABC type and susceptibility profile to antifungals in the first and second samples. One patient with 7 samples collected sequentially over 10 days showed 3 distinct strains, all discriminated by MLST. The first four samples were indistinguishable, the fifth and sixth were also indistinguishable but different from the first four and the seventh collected sample. Significant, the seventh strain recovered was the only *C. albicans* clade 2 isolate found in our total collection involving 61 patients (data not shown), although clade 2 is commonly found worldwide.

**Conclusions:** To the best of our knowledge, this is the first study describing a blood stream infection with 3 distinct *C. albicans* strains in the same patient within a short period of time.

**P523 An optimised RAPD protocol for rapid genotyping and local epidemiological mapping of *Clostridium difficile***

L. Green*, J. Rollason, T. Worthington, A.C. Hilton, P.A. Lambert (Birmingham, UK)

**Objectives:** To develop an optimised Random Amplified Polymorphic DNA (RAPD) PCR protocol for rapid and reproducible discriminatory typing and local epidemiological mapping of *Clostridium difficile*.

**Methods:** Two 10-bp primers were selected and used in separate amplification reactions to determine their ability to discriminate between different PCR ribotypes of *C. difficile*. Concentrations of deoxyribonucleotide triphosphates (dNTPs), primer and template DNA were titrated to determine optimal concentrations for reproducible and discriminatory amplification. Concentration of Magnesium Chloride (MgCl2), Potassium Chloride (KCl) and pH of the reaction buffer were also optimised. The amplification cycles used were as follows: 5 cycles of 4.5 minutes (94°C), 30 seconds (94°C), 2 minutes (97°C) and 30 seconds (94°C), 30 seconds (30°C) and 1 minute (72°C). Amplified products were size separated on a 2% agarose gel in 1× TAE running buffer (40 mM Tris-HCl, 1 mM EDTA, 0.1% (v/v) glacial acetic acid, pH 8). Following electrophoresis, amplicons were detected by Ethidium Bromide staining and viewed under UV light. Following RAPD optimisation, both primers were used to type a panel of control PCR ribotypes (001, 002, 005, 014, 015, 017, 023, 027, 064, 078, and 106); clinical isolates known to belong to the same ribotype were also tested. DNA preparations from selected isolates were prepared in duplicate for amplification in the same cycling reaction and at widely different times to determine reproducibility.

**Results:** The parameter having the greatest influence on the discriminatory capacity and reproducibility of RAPD was the reaction buffer; KCl having the greatest effect. The optimised RAPD protocol generated a unique profile for each distinct ribotype tested and clustered identical ribotypes together. Profiles were reproducible when generated from independently prepared reactions using different batches of reagents.

**Conclusion:** RAPD, when optimised effectively, has the ability to produce reproducible and stable amplification profiles. When applied to isolates which had been characterised previously by PCR Ribotyping RAPD demonstrated the same discriminatory capacity; isolates with unique ribotypes had unique RAPD types and those indistinguishable by ribotyping were also grouped together by RAPD. Optimised RAPD offers a rapid and cost-effective method for the epidemiological typing of *C. difficile*.

**P524 Genotypic characterisation of binary-toxin-producing *Clostridium difficile* strains**


**Background:** *Clostridium difficile* (CD) is an important cause of community and nosocomial diarrhea. In recent years, epidemic strains belonging to the 027 and 078 ribotypes have emerged. Both ribotypes are characterised by binary toxin production and deletions of tcdC gene (negative regulator of toxin production), the latter possibly related to increased toxin production.

**Objective:** To genotype the binary-toxin-positive CD strains (bin+) circulating in our hospital during the year 2007.

**Methods:** CD strains were cultured and identified by conventional microbiological methods. Toxins A and B were detected in isolates using an immunochromatographic method (ImmuCard, Meridian Bioscience). DNA was obtained from pure cultures using Chelex resin (Instagene matrix, BioRad). The tcdA gene (toxin A), tcdC gene (toxin B), and binary-toxin genes cdIA and cdIB were detected by PCR following methods previously described (Kato, 1991; Wolfgahan, 1994; and Stubbs, 2000, respectively). Bin + isolates were characterised by PCR-ribotyping (Bidel et al. 2000). Phylogenetic analysis of ribotyping profiles was conducted using BioNumerics software 5.0. The tcdC gene was amplified by PCR using the method described by Spigaglia and Mastrantonio (2002), sequenced by the Big Dye Terminator method, and detected in an ABIPrism 3100 automatic DNA sequencer (Applied Biosystems Inc.). Sequences were aligned using BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

**Results:** Seven hundred and forty three CD toxigenic strains were isolated from patients with CD associated diarrhoea during 2007. Eighty-eight isolates were bin + (62 patients) and all were also tox A+B+. The isolates were from 8 different ribotypes. Most CD isolates belonged to ribotype 078 (63 isolates from 45 patients). Only 6 isolates (2 patients) belonged to ribotype 027. The analysis of the tcdC gene revealed deletions of 18bp in 10 isolates (2 different ribotypes), 36bp in 5 isolates (2 different ribotypes), 39bp in 69 isolates (2 different ribotypes), and 54bp in 6 isolates (only 1 ribotype).

**Conclusions:** Twelve percent of our CD toxigenic isolates had binary-toxin genes. Ribotype 078 is a frequent cause of diarrhoea in our patients, representing 8% of toxigenic CD isolated at our institution during 2007.
Co-existence of multiple MLV A sub-types of Clostridium difficile PCR-ribotype 027 strains within fecal specimens

H.E. Tanner*, K.J. Hardy, P.M. Hawkey (Birmingham, UK)

Objectives: Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) for the nosocomial pathogen C. difficile has previously been shown to be capable of sub-dividing isolates of the epidemic strain PCR-ribotype 027. The aim of the study was to investigate whether MLVA typing could identify different subtypes of ribotype 027 within individual faeces specimens from cases of C. difficile infection (CDI) and to determine the impact this may have on the utility of MLVA as a typing method for outbreak situations.

Methods: Five isolates of PCR-ribotype 027 C. difficile were cultured from each of 39 faeces specimens from patients with CDI and typed with MLVA.

Results: Twenty two specimens tested contained at least two different, but closely related, MLVA profiles. Five isolates yielded isolates with MLVA profiles over five summed tandem repeats different from other isolates from the same specimen.

Conclusion: These observations agree with previously published studies using different typing methods showing that multiple types of C. difficile can co-exist in a single faeces specimen. These differences may have the potential to obscure epidemiological links between cases of CDI.

First nationwide estimates on incidence and case fatality of Clostridium difficile-associated infections

O. Lyytikäinen*, M. Snellman, S. Kortila, S. Ibrahim, A. Virolainen-Julkunen (Helsinki, FI)

Objectives: In 2008, C. difficile were included in the national surveillance, and clinical microbiology laboratories were asked to send C. difficile isolates from severe cases and persistent outbreaks to the reference laboratory for genotyping. We analyzed the first 9-month surveillance data to assess the incidence and case fatality of CDI, and to detect regional differences in CDI epidemiology.

Methods: All laboratories reported all C. difficile findings (positive culture and/or toxin production) from stools to the National Infectious Disease Register. Each notification included specimen date, each individual’s national identity code, date of birth, sex, and place of residence. Within this information and a 3-month time interval, multiple notifications of the same person were merged as a single episode. The dates of deaths were obtained from the Population Information System. An episode of CDI was defined as detection of C. difficile toxin from stools of persons aged >2 years from January 1, 2008 to September 30, 2008. PCR ribotyping was performed according to the protocol of the Anaerobe Reference Unit in Cardiff, using the Cardiff-ECDC collection of different C. difficile PCR ribotypes as reference. When a local outbreak was suspected, also pulsed-field gel electrophoresis was performed.

Results: In total, 4571 episodes of CDI among 4411 individuals were identified; 3169 (69%) occurred in persons aged >64 years and 2707 (59%) in females. The overall incidence was 11.5 per 10,000 population (range by regions, 5.5–19.4). The incidence increased by age and was highest in persons aged >84 years (128.2). Of the CDI episodes, 202 (4.4%; range by regions, 0–8.9%) lead to death within 7 days and 526 (11.5%; range by regions, 0–16.2%) within 30 days. The 7- (7.4%) and 30-day (20.8%) case fatality was highest in persons aged >84 years. In total, 223 (5%) isolates from 12/20 regions were sent for genotyping: 108 (48%) were of PCR ribotype 027, 28 (13%) of 001, and 10 (4%) of 002; among the rest of the isolates, some 30 distinct PCR ribotype profiles were identified, including 023, 045 and 078. The isolates of PCR ribotype 027 came from 6/12 regions. The non-027 ribotypes were equally common among severe cases as the 027.

Conclusions: Our study showed the first nationwide population-based estimates on incidence and case fatality of CDI. The major regional variations may be due to differences in diagnostic activity or spread of hypervirulent PCR ribotypes.

A rapid and simple identification of Clostridium difficile PCR ribotype 027 by loop-mediated isothermal amplification method

H. Kato*, Y. Ito, Y. Arakawa (Tokyo, Gifu, JP)

Objectives: A number of outbreaks caused by Clostridium difficile PCR ribotype 027 have been documented in North America and Europe. The emerging strain was reported to cause more severe disease. Earlier recognition of PCR ribotype 027 might be beneficial in countries where the type is epidemic as well as in countries where the type is not currently predominant. We established and evaluated a loop-mediated isothermal amplification (LAMP) method for identification of PCR ribotype 027.

Methods: A total of 85 C. difficile isolates recovered from symptomatic patients admitted to hospitals in Japan were used. The collection of PCR ribotype 027 isolates for the previous collaborative typing study (Killigore G. et al. 2008. J Clin Microbiol) was also included. Isolates were analyzed by two typing systems, PCR ribotyping and surface layer protein A gene (slpA) sequence typing. Four primers derived from the sequence of the slpA gene of PCR ribotype 027 were used for the LAMP assay to identify PCR ribotype 027. The increased turbidity of amplified products was monitored by a real-time turbidimeter.

Results: The 85 isolates tested were typed into 18 PCR ribotypes including PCR ribotype 027. The isolates representing the 18 PCR ribotypes were tested for slpA of PCR ribotype 027 by LAMP. The slpA gene of PCR ribotype 027 was detected by LAMP in all PCR ribotype 027 isolates examined; no amplification products were obtained in isolates belonging to other PCR ribotypes. DNA was extracted directly from a stool specimen of a patient who suffered from pseudomembranous colitis caused by PCR ribotype 027 strain. In addition, the stool specimen of this patient was cultured in cooked meat medium overnight, followed by DNA extraction by a simple boiling method. The slpA sequence of PCR ribotype 027 was detected by LAMP in the both DNA extracts.

Conclusion: The LAMP assay detecting the slpA sequence of PCR ribotype 027 appears to be a valuable tool for the rapid identification of this type. This method could be feasible to detect PCR ribotype 027 C. difficile in stool specimens.

Comparison of molecular and susceptibility characteristics of CA-MRSA and HA-MRSA among hospital-admitted patients in two main cities of Iran: 1-year study

M. Moghadami*, M. Mardani, M. Amini (Shiraz, Tehran, IR)

Objective: Methicillin-resistant Staphylococcus aureus (MRSA) has traditionally been considered a health care-associated pathogen in patients with established risk factors. However, MRSA has emerged in patients without established risk factors. To characterise epidemiological and microbiological characteristics of community-associated MRSA (CA-MRSA) cases compared with health care-associated MRSA (HA-MRSA) cases in our country this survey was done.

Method: Prospective cohort study was done for patients with MRSA infection identified at 4 medical universities’ hospitals in Tehran (capital of Iran) and main hospital in Shiraz (main city in South of Iran) from December, 2007 through September, 2008, comparing CA-MRSA with HA-MRSA cases. Clinical infections associated with either community-associated or health care-associated MRSA, microbiological characteristics of the MRSA isolates including susceptibility testing (MIC Determination by broth method for clindamycin, rifampin, doxycyclin, trimethoprim-sulfamethoxol, tetracycline, ciprofloxacin), and staphyloccocal SCCmec genes type was done (by multiplex PCR) were determined.

In our study, epidemic strains of ribotype 027 were detected in only 2 patients. All the bin + CD detected had deletions in the tcdC gene.
**Results:** Of 109 documented MRSA infections, 15 (12%) were community-associated and 94 (85%) were health care-associated. The staphylococcal cassette chromosome mec (SCCmec) types and antimicrobial susceptibility patterns of all MRSA strains were determined. Although community-associated MRSA isolates were more likely to be susceptible to antimicrobials, most community-associated infections were initially treated with antimicrobials to which the isolate was nonsusceptible. Most frequent SCCmec in our hospitals was SCCmec-I (56.9%) and least one was SCCmec-III (10.1%) and relevant SCCmec with community was type IV.

CA-MRSA strain were significantly more susceptible to above antibiotics in comparison to HA-MRSA, as presented in the following table:

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Number (%)</th>
<th>HA-MRSA</th>
<th>CA-MRSA</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>68 (72)</td>
<td>5 (33)</td>
<td>73 (67)</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>60 (63)</td>
<td>4 (26)</td>
<td>64 (58)</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>73 (77)</td>
<td>8 (53)</td>
<td>81 (74)</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>63 (67)</td>
<td>5 (33)</td>
<td>68 (62)</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>78 (83)</td>
<td>6 (40)</td>
<td>84 (77)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>73 (77)</td>
<td>9 (40)</td>
<td>79 (72)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>58 (60)</td>
<td>4 (26)</td>
<td>62 (57)</td>
<td>0.011</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:** Our study, as we know, was first study in Iran about differentiation of different characteristic of CA-MRSA and HA-MRSA. Our study, as we know, was first study in Iran about differentiation of different characteristic of CA-MRSA and HA-MRSA. In two MRSA strains, it was not possible to detect the presence of the mecA gene.

**References:**

**P531** Molecular and phenotypic characters of methicillin-resistant *Staphylococcus aureus* bloodstream isolates from Taiwan

W.Y. Wang*, J.J. Lu, G.W.S. Tsai (Miao-Li, Taichung, Taipei, TW)

**Objectives:** *Staphylococcus aureus* is a major pathogen responsible for bacteraemia and sepsis with extremely high mortality. Methicillin-resistant *S. aureus* (MRSA) has emerged since 1960s and increased to more than 70% in Taiwan. Limited MRSA clones circulating worldwide have been identified. We intend to illustrate the molecular and phenotypic characters of MRSA blood stream isolates from Taiwan.

**Methods:** MRSA isolates from patients with bacteraemia were retrieved from database of the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). Antibiotic susceptibility tests were performed with disc diffusion and inducible macrolide-lincosamide-streptogramin resistance (MLSBi) were validated with D test. Molecular types of MRSA blood stream isolates were assigned by PCR-based typing methods of accessory gene regulator (agr), staphylococcal cassette chromosome mec (SCCmec), direct repeat units (DRUs), and multilocus sequence typing (MLST). Gene encoding Panton-Valentine leukocidin (pvl) was also amplified by PCR and identified by electrophoresis.

**Results:** Totally 160 non-duplicate MRSA blood stream isolates were selected from the database of 9 medical centers. All isolates were identified as MRSA with presence of mecA. There were 6 (3.8%), 117 (73.1%), 22 (13.8%), 1 (0.6%), 11 (6.9%), and 3 (1.9%) isolates assigned as SCCmec type II, III, IV, V, VT, and non-typable strains, respectively. Most MRSA isolates (150, 93.8%) belonged to agr type I and the other 10 isolates belonged to agr type II (6.2%). Significant association was found between 4 DRUs copies number with agr type II (6/10, p < 0.001), 14 DRUs copies number with SCCmec III (75/76, p < 0.001), and 9 DRUs copies number with SCCmec IV (18/22, p < 0.001). There were 13 (8.1%) isolates assigned as positive MLSBi phenotype and no significant association of MLSBi with SCCmec type or DRUs copies number (8.1%) isolates assigned as positive MLSBi phenotype and no significant association of MLSBi with SCCmec type or DRUs copies number (p = 0.10) was noted. Forty-one MRSA isolates were typed with MLST and 5 major clusters were identified (9 with ST59 and 1 with ST338, 17 with ST239 and 6 with ST241, and 6 with ST5). Only 14 isolates (8.8%) were positive for pvl and were significantly associated with SCCmec VT (11 isolates, p < 0.001). Resistance to 4 or more antibiotics was noted mostly in SCCmec III but rarely in SCCmec IV and VT.

**Conclusions:** Two major clones of SCCmecII-ST239-argl-DRUs14 and SCCmecIV-ST59-argl-DRUs9 were predominant in MRSA blood isolates from Taiwan. MLSBi and pvl gene were not prevalent in MRSA blood isolates.

**P532** Epidemiological typing of methicillin-resistant *Staphylococcus aureus* isolates from India and Pakistan

S. Shahir, K. Hardy*, W. Abbasi, C. McMurray, S. Malik, P. Hawkey (Birmingham, UK; Islamabad, PK)

**Objectives:** To gain a greater understanding of the epidemiology of MRSA in the subcontinental regions of India and Pakistan.

**Methods:** Sixty MRSA isolates were obtained from three regions; Pakistan (2) and India (1). All isolates were confirmed as MRSA using biochemical tests and typed using a range of genotypic methods. Detailed epidemiological relationships were identified using pulsed field gel electrophoresis (PFGE) and staphylococcal interspersed repeats (SIRU), whilst the overall global epidemiology was studied using the restriction modification (RM) method and multi locus sequence typing (MLST).

**Results:** All isolates were typable by PFGE, SIRU and RM assignment. 57/60 isolates were all closely related, all belonging to CC8, differing at only one locus using SIRU and clustering within 67% relatedness by PFGE. Within CC8, SIRU typing sub-divided the isolates into 12 different profiles all of which were closely related. Two of the SIRU profiles were present in isolates from both India and Pakistan, whilst nine were distinct to Pakistan and one to India. If the strict criterion of one band difference was applied to the PFGE profile a total of 24 different types were identified within the CC8 isolates. Unlike the SIRU profiles where the same profiles were present in both Pakistan and Indian isolates, all PFGE profiles were distinct between the two countries. MLST typing of ten CC8 strains with diverse SIRU and PFGE patterns revealed eight belonged to ST239, one to ST113 and ST85 respectively. The 3 isolates with a different clonal complex all belonged to CC30 and were all from the same hospital in Pakistan.

**Conclusion:** Epidemiological typing of strains from three distinct locations in India and Pakistan reveals that ST239 is the predominant ST type and could be presumed to have been present for some time. SIRU and PFGE differentiated within ST239 demonstrating their utility and the importance of using epidemiological typing methods with a high degree of discrimination when investigating clusters and outbreaks within these countries.

**P533** The clonal structure of PFGE non-typeable methicillin-resistant *Staphylococcus aureus* in the Netherlands

J.W. Huisjens*, T. Bosch, M.G. van Santen-Verheuvel, E. Spalburg, M. Heck, G.N. Plaatser, M. van Luit, A. Haenen, A.J. de Neeling (Bilthoven, NL)

**Objectives:** In the Netherlands, the National Institute for Public Health and the Environment (RIVM) serves as the national reference centre for surveillance of MRSA. Recently, animals such as pigs and calves were identified as possible MRSA reservoirs. MRSA isolates (either human or animal), referred to as NT-MRSA, related to livestock belong to a relatively new CA-MRSA clonal lineage, ST398. The present study gives an overview of the human NT-MRSA isolates in the Netherlands in 2007.

**Methods:** The molecular characteristics of NT-MRSA and the clonal structure of the isolates was determined by Panton-Valentine leukocidin (PVL), staphylococcal cassette chromosome mec (SCCmec) typing, and multilocus sequence typing (MLST). For the SCCmec typing the multiplex PCR method of Kondo et al. was used.

**Results:** In 2007, a total of 793 human MRSA isolates were non-typeable by PFGE. Three isolates were PVL positive. Spa typing revealed 27 different spa types, all related to each other. The two most prevalent spa types were 1011 and 1108. SCCmec typing results of 300 NT-MRSA isolates showed SCCmec type IV, type V and unknown type(s). Surprisingly, all isolates with spa type 1108 were either SCCmec type V or could not be typed, no SCCmec type IV was found. One isolate per spa type was subjected to MLST. All isolates belonged to the ST398 clonal lineage.

**Conclusion:** The clonal lineage ST398 is not specifically found in pigs anymore, but has also been found in other animals, serving as MRSA reservoirs. Whether the number of NT-MRSA will increase even further remains to be seen. Surprisingly, independent of the host (animal or human) the typing results of NT-MRSA isolates showed the same clonal origin.

**P534** dru-typing.org: an Internet resource for the sequence-based typing of methicillin-resistant *Staphylococcus aureus* and the analysing the hypervariable mec-associated direct repeat unit

R.V. Goering* (Omaha, US)

**Objectives:** Variable-number tandem repeat (VNTR) sequences have found important use in the epidemiological typing of problem bacterial pathogens. With *Staphylococcus aureus*, sequence analysis of the polymorphic X region of the protein A gene (spa), coupled with a uniform system of nomenclature, has resulted in a robust method for epidemiological analysis. In methicillin-resistant *Staphylococcus aureus* (MRSA), the direct-repeat unit (dru) VNTR region adjacent to IS431 in SCCmec has also proved useful in the epidemiological analysis of highly uniform epidemic strains (e.g., EMRSA15 and -16) and in tracking the horizontal movement of SCCmec. Recently, more efficient use of dru typing has been facilitated by a proposed uniform system of nomenclature (Goering et al., 2008; Clin. Microbiol. Infect. 14:646–659). However, optimum use of this typing approach requires a convenient means where newly generated data can be cataloged and compared in an internationally shared database.

Results: Based on the newly published nomenclature, the dru-typing.org website allows investigators to enter user generated 40-bp repeat sequences which are then searched against the current database of dru repeats and identified, if known. Specific combinations of repeats may also be queried against the database and, if recognized, the resulting dru type will be identified. New dru repeat and/or dru type chromatograms can be submitted online for verification and inclusion into the database. At present, the growing database contains 51 different dru-repeat sequences and 137 dru types which can be downloaded for off-line reference.

Conclusions: Dru-typing.org represents the first freely-available Internet-accessible database for collecting and harmonising dru-repeat and dru-type sequences. The website provides an interface which should assist in standardising and facilitating the use of dru typing as a tool in the epidemiological and evolutionary analysis of MRSA strains.

P535  Stability of spa types from multiple MRSA isolates from the same persons
K. Boye*, S. Rohde, H. Westh (Copenhagen, DK)

Objectives: Sequencing of the repeat region of the Staphylococcus protein A gene (spa) has since 2003 been used in our department to monitor outbreaks of MRSA, follow routes of transmission and study the evolutionary relationship of MRSA. This typing system works well on the MRSA population in Copenhagen (Bartels et al. 2007). The aim of this study was to examine how often and how fast the spa region of MRSA changes, both over time and from different body sites.

Methods: All MRSA isolates including duplicate isolates from the same person, from 2004–7, in Copenhagen, were spa typed by PCR and sequencing as previously described (Bartels et al. 2007).

Results: From 2004–2007 we received between two and 35 individual MRSA samples from 335 individuals, a total of 1598 MRSA including 1263 duplicate isolates. 36% of the persons had two MRSA samples, 31% had more than five. One person contributed with 35 MRSA isolates. 66 spa types and 24 NTs were found in the 1598 isolates. spa types occurred between one and 737 times (024: 46% of all isolates). 41% of the spa types were only seen one or two times.

26 persons (8%) exhibited more than one spa type. Seventeen (5%) had MRSA with spa types that could evolve from each other, most often by deletion or duplication of repeats. Nine had more than one MRSA judged by the presence of very different spa types where the variation could not be explained by mutational changes. No more than two spa types were retrieved from one person. In eight of the 17 cases, both spa types were found at the same time. In nine cases a period between 1 and 20 months passed between the occurrences of the two types (median 10 months). Often the most common spa type was recorded both before and after the less common one.

Conclusions: The spa-repeat region is very stable over time. Only in 5% of the 335 persons actual evolution/change of spa type was found. It did not seem to be a process at one spa type replacing another but more likely the co-existence of spa type variants with a common origin.

Reference(s)

P536  Clonal enrichment of integrated resistance plasmid-containing Staphylococcus aureus in a burn centre associated with persistent carriage among healthcare workers

Objectives: Burn wound surfaces are generally infected by S. aureus, but the reservoirs and transmission routes remain to be elucidated.

The genetic population structure of serial S. aureus isolates obtained from patients and healthcare workers (HCWs) in a burn centre was investigated. We assessed the frequency of auto- versus exo-infection and established a model describing import and local persistence of S. aureus clones.

Methods: Three populations of S. aureus isolates were collected (2001–2005) and typed by PFGE. Population I comprised 375 strains from HCWs, Population II harboured 586 nosocomially acquired strains from burn wounds. Population III involved 202 strains from patients at admission. Comparative genome hybridisation (CGH) was performed for endemic versus incidental S. aureus strains.

Results: The diversity index for Population III was significantly higher than those for Populations I and II. Three PFGE-types were clearly endemic among HCWs and nosocomially acquired S. aureus strains. CGH revealed that endemic strains possessed an integrated plasmid encoding resistance to heavy metals.

Conclusion: Genetic diversity for S. aureus strains circulating in the burn centre was lower than that of strains in the open community. Apparently, endemic S. aureus clones have a superior potential to colonise burns which may be associated with their heavy metal resistance in an environment where silver and cerium containing antibiotics are the most used.

P537  Spa versus phage typing – utility for analysing diverse MSSA in the UK
M.J. Ellington*, S. Hashim, M. Gunner, B.D. Cookson, A.M. Kearns (London, UK)

Objectives: For more than 30 years phage typing has been used to type S. aureus. The method assays the pattern of phenotypic susceptibility to a panel of S. aureus bacteriophages. In recent years genotypic, DNA sequence based, spa typing has been adopted internationally. We aimed to determine the correlation between spa typing and phage typing amongst genetically diverse, disease associated, MSSA from around the UK.

Methods: Diverse S. aureus isolates (n=170) from around England, Wales and Northern Ireland, referred to the national Staphylococcus Reference Unit from September 2006 to 2007, were selected for testing based upon their phage type at 100 x routine test dilution (RTD) and their toxin gene profile. Additional phage typing was carried at routine test dilution (RTD) and DNA sequencing of spa amplicons was performed according to Harmsen et al. (2003). Phage types were assigned into groups II, group 95, group 94/96 and mixed according to their phage susceptibility profiles. Data was collated and analysed using BioNumerics software.

Results: The spa type correlated phage type for 132/170 (78%) S. aureus isolates, and in 81% of cases individual spa types mapped to a single phage-type. Phage-type could be correlated with spa inferred MLST clonal complexes for 157/170 isolates, with at least 15 CCs represented amongst the isolates. Isolates from phage group II belonged to CCs 15 or 121 in 79% of cases, 93% of phage group 94/96 isolates were CC25 or 152 and 10/11 (91%) phage type 95 isolates were CC45. Isolates belonging to the “mixed” phage-type group were more genetically diverse and comprised isolates from at least 8 different CCs including CC1, 5, 8, 12, 30 and 59. Amongst 28 isolates that were non-phage typeable, nine had spa types related to CC22 isolates of the same lineage as the dominant MRSA strain in the UK (EMRSA-15). The remaining 19 non-phage typeable isolates were genetically diverse suggested by spa typing and belonged to at least 8 different CC’s.

Conclusions: Amongst MSSA found in the UK, spa typing accurately predicted phage-type in over three quarters of a panel of 170 diverse isolates, and was able to predict the genetic relatedness and diversity amongst phage non-typeable isolates. Marked associations between some phage groups and MLST CCs was noted (CC15 and 121 with group II) indicating the broad comparability of spa data with previously collected phage data, for some phage groups at least.
**Staphylococcus aureus genotypes in patients with cystic fibrosis**

K. Semczuk*, K. Dzierzanowska-Fangrat, H. Dmenska, D. Dzierzanowska (Warsaw, PL)

**Objectives:** The aims of this study were to determine: (a) identity of *S. aureus* strains isolated from airways of cystic fibrosis (CF) patients; (b) *S. aureus* genotype typical for CF patients; (c) duration of airways colonisation.

**Methods:** From 1998 through 2005 sputum and throat swabs were collected 2–3 times a year from 33 CF patients (age range 6 months – 17 years). A total of 392 specimens were obtained (mean 12/patient). Identifications and susceptibility testing were performed using standard procedures. PFGE method with digestion with Smal RE was used for genotyping. Restriction profiles were analysed using Molecular Analyst Fingerprinting Plus Programme.

**Results:** A total of 297 *S. aureus* isolates (mean 9/patient) that belonged to 76 different genotypes were collected. Majority, i.e. 58 PFGE types showed a tendency for persistent colonisation (3–8 years). Only 6% of strains were isolated on a single occasion. In 27% of patients isolated strains were identical during the whole study period. 12% of patients were simultaneously colonised by 2 or 3 genotypes of *S. aureus* with a predominance of a single genotype. In 9% of children a long-lasting single genotype was replaced overtime by another persistent genotype. In the remaining 52% of patients various *S. aureus* genotypes were isolated on subsequent visits. MRSA strains were found in 9% of children. These isolates belonged to 3 genotypes showing different susceptibility profiles.

**Conclusions:** The airways of CF patients can be simultaneously colonised by a few genetically and phenotypically divergent *S. aureus* strains capable of long-lasting persistence. No *S. aureus* genotype typical for CF patients was found.

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**Molecular virology**

**P539** Measuring human immunodeficiency virus type 1 RNA loads in dried blood spot specimens using NuclISSENS EasyQ HIV-1 v2.0

P van Deursen*, A. Verhoeven, P. de Bie, J. de Jong (Bostel, NL)

**Background:** The amount of HIV-1 RNA measured in plasma is one of the key parameters for monitoring anti-viral treatment responses in HIV-1 infected individuals. Accurate viral load measurement depends strongly on sample stability. In remote areas, sample collection sites can be located far from test sites, meaning that sample stability and thereby accurate measurement of the clinical state of the treated individual is at risk. To circumvent this possible risk, dried blood spot (DBS) testing is proposed as alternative for plasma testing.

**Objectives:** The aim of this study is to determine HIV-1 RNA stability in DBS using different storage conditions.

**Methods:** A total of 5, 10, 4 and 5 EDTA whole blood samples obtained from HIV-1 RNA negative individuals were spiked with HIV-1 RNA: 0 VQA cps/ml, 700 VQA cps/ml, 21,000 VQA cps/ml and 70,000 VQA cps/ml, respectively. For these samples, 50 ml spots were made on paper (ProteinsaverTM 903® Card, Whatman) and dried 3–48 hours. Next samples were stored at different temperatures (~20°C, 5°C, RT, 37°C, and 55°C). Samples stored at 37°C were subjected first to shipment simulation; 5 days 37°C (of which 8 hours high humidity at 37°C and 8 hours high humidity at 55°C), 3 days 5°C, 5 days −20°C, and subsequently 5 days 37°C (of which 8 hours high humidity at 37°C and 8 hours high humidity at 55°C). High humidity conditions were tested at 37°C and 55°C. After storage HIV-1 RNA levels were measured using two spots (0.1 ml blood) and NuclISSENS EasyQ HIV-1 v2.0.

**Results:** For the high input samples no or limited reduction in HIV-1 RNA levels (≤ 0.30 log10) was observed for all conditions tested with one exception (6 weeks 37°C at high humidity). For the low input sample (70 VQA copies/extraction) the detection rate was > 80% for all conditions tested, with an overall detection rate of 244/250 (97.6%). Sample stability was demonstrated for a period of 9 weeks at 37°C (with and without including shipment simulation), 3 weeks high humidity at 37°C, 1 week high humidity at 55°C, 3 weeks 4°C, 1 week −20°C, and 5 months room temperature.

**Conclusion:** HIV-1 RNA stability in DBS was demonstrated for several conditions including 5 months room temperature, shipment simulation, and storage at 37°C for 9 weeks. Instability was observed after 6 weeks 37°C high humidity. The results support the use of DBS specimens for accurate viral load measurements after transport and storage when taken these limitations into account.

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**P540** Measuring HIV-1 RNA stability in dried blood spot specimens using NuclISSENS EasyQ HIV-1 v2.0

P van Deursen*, A. Verhoeven, P. de Bie, J. de Jong (Bostel, NL)

**Background:** The amount of HIV-1 RNA measured in plasma is one of the key parameters for monitoring anti-viral treatment responses in HIV-1 infected individuals. Accurate viral load measurement depends strongly on sample stability. In remote areas, sample collection sites can be located far from test sites, meaning that sample stability and thereby accurate measurement of the clinical state of the treated individual is at risk. To circumvent this possible risk, dried blood spot (DBS) testing is proposed as alternative for plasma testing.

**Objectives:** The aim of this study is to determine HIV-1 RNA stability in DBS using different storage conditions.

**Methods:** A total of 5, 10, 4 and 5 EDTA whole blood samples obtained from HIV-1 RNA negative individuals were spiked with HIV-1 RNA: 0 VQA cps/ml, 700 VQA cps/ml, 21,000 VQA cps/ml and 70,000 VQA cps/ml, respectively. For these samples, 50 ml spots were made on paper (ProteinsaverTM 903® Card, Whatman) and dried 3–48 hours. Next samples were stored at different temperatures (~20°C, 5°C, RT, 37°C, and 55°C). Samples stored at 37°C were subjected first to shipment simulation; 5 days 37°C (of which 8 hours high humidity at 37°C and 8 hours high humidity at 55°C), 3 days 5°C, 5 days −20°C, and subsequently 5 days 37°C (of which 8 hours high humidity at 37°C and 8 hours high humidity at 55°C). High humidity conditions were tested at 37°C and 55°C. After storage HIV-1 RNA levels were measured using two spots (0.1 ml blood) and NuclISSENS EasyQ HIV-1 v2.0.

**Results:** For the high input samples no or limited reduction in HIV-1 RNA levels (≤ 0.30 log10) was observed for all conditions tested with one exception (6 weeks 37°C at high humidity). For the low input sample (70 VQA copies/extraction) the detection rate was > 80% for all conditions tested, with an overall detection rate of 244/250 (97.6%). Sample stability was demonstrated for a period of 9 weeks at 37°C (with and without including shipment simulation), 3 weeks high humidity at 37°C, 1 week high humidity at 55°C, 3 weeks 4°C, 1 week −20°C, and 5 months room temperature.

**Conclusion:** HIV-1 RNA stability in DBS was demonstrated for several conditions including 5 months room temperature, shipment simulation, and storage at 37°C for 9 weeks. Instability was observed after 6 weeks 37°C high humidity. The results support the use of DBS specimens for accurate viral load measurements after transport and storage when taken these limitations into account.

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**P541** Multicentre evaluation of the Versant® HIV-1 RNA 1.0 Assay (kPCR) with the Versant™ kPCR molecular system


**Objectives:** The Siemens VERSANT® HIV-1 RNA 1.0 Assay (kPCR) is a reverse transcription kinetic polymerase chain reaction (kPCR) method for quantifying human immunodeficiency virus type 1 (HIV-1) RNA in human plasma, using the VERSANT kPCR Molecular System – a semi-automated system combining a fully automated sample preparation module and a fully automated amplification and detection module. This study examined the performance characteristics of the assay, and a method comparison was conducted between the VERSANT HIV-1 RNA 1.0 Assay (kPCR) and the Abbott RealTime® HIV-1 assay.
Comparison of commonly used real-time PCR methods to quantify HIV-1 DNA in lymphomonocytes from infected patients

**Objectives:**
- Different PCR methods have been widely used to quantify HIV-1 DNA. The clinical use of HIV-1 DNA may be limited by the lack of an international standard to calibrate the different methods. In addition, genetic variability of HIV-1 subtypes may profoundly influence the accuracy of the assays. The aim of the study was to evaluate three non-commercially common used real-time PCR to quantify proviral HIV DNA in lymphomonocytes from infected patients.

**Methods:**
- Parallel evaluation with 3 assays to quantify HIV-1 DNA targeting pol, gag and LTR regions was performed in 93 lymphomonocytes from 43 patients. All the patients harboured HIV-1 B subtype and were in the chronic phase. Twenty-three patients were successfully treated with HAART and underwent CD4-guided treatment interruption; the remaining 20 patients included subjects with virological failure because of multiple-drugs resistant HIV strains. An additional real-time PCR targeting a cellular gene (hTERT) was done to refer the HIV-1 DNA copies to 1 million lymphomonocytes. Full-length sequencing of the virus present in a representative patient was performed by massive parallel pyrosequencing (GS-FLX platform, Roche).
- A statistically significant difference between the mean values of HIV-1 DNA in clinical samples obtained by pol, gag and LTR real-time PCR was observed. Only the LTR-targeting PCR was able to detect HIV-1 DNA in all the samples from all the patients, with a number of copies always at least 1 Log higher than that obtained by the two other methods. A stronger correlation between viraemia (HIV-1 RNA) and proviral load was found when HIV-1 DNA was measured with LTR real-time PCR as compared to pol real-time PCR, while the correlation was not significant with gag real-time PCR. Full-length sequencing of the virus, present in a representative patient, clearly showed the simultaneous presence of multiple mismatches in the primers and probes of pol real-time PCR. Conversely, only 1 mismatch was observed in one of the primers of gag real-time PCR.

**Conclusions:**
- The data strongly suggest that HIV-1 DNA quantification may be very different, depending on the genome target region. The possible use of HIV-1 DNA as a marker to predict disease progression and treatment outcome in infected patients depends on standardisation of the laboratory methods to minimise variability in genome recognition by the different molecular tools.
identify 52 different HPV types, among which HPV-16 was by far the most commonly detected (6.2% of all specimens), followed by HPV-6 (2.6%) and HPV-66, HPV-58, and HPV-53 (2.3% each). HPV-18 was detected in 0.6% of samples and HPV-11 in 0.3%. Overall, high-risk HPV types accounted for 42.6% of detected HPVs, probably high-risk HPVs for 16.6%, low-risk HPVs for 22.2%, and undetermined-risk HPVs for 18.6%. Pap cytology results were available for a subgroup of 1,088 women: 31% with a negative Pap test, 27% with ASC-US, 39% with LSIL, and 2% with HSIL or ASC-H. Prevalence of HPV DNA detection increased with severity of cytology, from 39% in negative samples to 100% in HSIL. HPV-16 and/or HPV-18 were present in 7% of normal cytology samples, in 5.7% of ASC-US, in 6.3% of LSIL, and in 27% of HSIL/ASC-H. Among high-risk HPVs, HPV-58 and HPV-31 were the most commonly identified types in high-grade lesions, besides HPV-16. Classification of HPVs into species showed that species 9 was the most represented (37% overall; 56% in HSIL), followed by species 3 (19%) and 6 (16%). Species 7, which includes HPV-18 and HPV-45 among other HPVs, was poorly represented in our population.

**Conclusions:** These data provide knowledge about the prevalence and type distribution of HPV in the Northeast of Italy and indicate that HPV-16/18 VLP vaccination is expected to reduce the burden of HSIL. Prevalence of HPV high-risk genotypes 16 and 18.

**Methods:** A six level reproducibility panel was prepared in PreservCyt media containing purified plasmid DNA with the cloned HPV genotype of interest and a positive control (negative control was HPV-18) and HCT-15 cells (source for the Beta-globin gene). The panel levels ranged from zero to titers seen in HPV-infected clinical specimens. Each of the three study sites performed multiple panel runs on the cobas 4800 system, where each run totaled 84 assays (14 replicates of each of 6 levels) plus a set of positive and negative controls. Reproducibility and precision were assessed with a linear mixed effects model, while sensitivity to the HPV titer was assessed using binomial regression.

**Results:** Comparable quality and technical precision were observed among all three participating laboratories for clinically relevant concentrations of HPV. For all runs, the positive and negative controls for the plate (one of each per plate) produced the correct results. No false positives were observed in any replicate. The prototype cobas® 4800 HPV test system shows good between-lab reproducibility and within-run precision for both HPV16 and HPV18.

**Conclusion:** The prototype cobas® 4800 HPV test system shows good between-lab reproducibility and within-run precision for both HPV16 and HPV18.

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**References**


**Objectives:** HPV persistence and progression has been recognized as strongly associated to anal as well as to cervical carcinoma; however, epidemiologic data have shown no reduction in the incidence of anal cancer so far, probably due to an increase in high-risk sexual behaviours. Besides high-risk groups, HPV infection in the anal canal occurs in immuno-competent individuals even in the absence of anal intercourse. To date, the role of HPV-type specific infection in anal lesions in women have been addressed in a few studies. Hence, the aim of this study was to monitor female anal infection, in terms of prevalence of HPV genotypes and type-specific viral load.

**Methods:** The presence of specific HPV genotypes in anal and cervical brushings was determined by two different PCR assays followed by sequencing, a method that allows the identification of a wide range of HPV types. Type-specific viral load was measured using a quantitative real-time PCR fluorogenic assay with TaqMan probes and primers designed for 14 HPV genotypes in the E6 genomic region.

**Results:** Anal brushings were collected from 12 HIV-positive and 40 HIV-negative women attending a proctology clinic. The prevalence of high-risk HPVs in anal specimens was higher than in cervical samples (67% vs. 25%). HPV genotypes detected in anal samples were: HPV 16 (4 cases), HPV 6 (3 cases), HPV 31 and HPV 53 (2 cases each), and HPV 62, 66, 74 and 84 (1 case each). Considering the women for whom anal and cervical cells were concurrently obtained, anal HPV was more common than cervical HPV in HIV-positive (63% vs. 37%) women; conversely, only about 10% of HIV-negative women harboured anal HPV infection vs. 37% of cervical HPV infection. The simultaneous presence of the same genotype occurred only in 2 out of 8 HPV positive women with both anal and cervical samples. The analysis of the association between grade of lesions, immune status and HPV load is in progress.

**Conclusion:** Our study confirmed the high prevalence of HPV anal infection in HIV-positive female patients. The distribution of high-risk HPVs in anal samples supported the need to develop HPV screening programs in anal brushings.
Novel mutation (A427S) in the CMV UL97 gene associated with resistance to ganciclovir

A. Anton, V. Dimoca, T. Pumarola, C. Cervera, L. Linares, A. Moreno, F. Cofán, M.T. Jiménez de Anta, M.A. Marco* (Barcelona, ES)

Introduction: Cytomegalovirus (CMV) infection is the leading viral cause of morbidity and mortality in kidney transplant recipients. Mutations in the CMV UL97 gene product, which confer ganciclovir (GCV) resistance, are well known but some unusual sequence changes observed in specimens from treated subjects remain uncharacterised.

Patient and Methods: We report a male who underwent a kidney transplant (D/R+) and where we detected on posttransplant day 111 a CMV resistant strain to GCV, after treatment for 5 weeks. The patient was monitored for CMV infection after transplantation by CMV antigenemia and viral load. When antiviral resistance was suspected, mutation screening by PCR sequencing of CMV UL97 and UL54 was performed from plasma samples. The amplified regions included almost all of the known resistance mutations. Derived sequences of each isolate were aligned with the strain AD169 reference sequence and amino acid differences were compared with previously published.

Results and Conclusions: Nucleotide sequence of the UL54 and UL97 of the clinical isolate showed 5 amino acid substitutions (N658S, S655L, N989D, S897L and A885T) in UL54, which were polymorphisms as already described, and 5 mutations (A427S, C428M, I429H, D430A, Y432N) in UL97. To our knowledge these mutations in UL97 of unknown significance have not been described yet. The codon 427 is outside the region with well-known mutations (codons 460, 520, and 590–607) related to GCV resistance. A change in the codon 427 (A427V) was already reported and phenotyped in a BAC recombination system as GCV sensitive (Martin M et al, 2006). In case we had been able to isolate this strain, the sensitivity test to GCV by phenotyping assay would have been interesting. As we did not detect these UL97 mutations from samples collected just in the beginning of the GCV treatment, we think that these may be associated with resistance to GCV. The antiviral treatment in this patient was changed to foscarnet with good virological response. Definitive conclusions about the role that such mutations play would require a marker transfer of the mutated UL97 region in a known susceptible CMV strain and the corresponding IC50 value by recombinant phenotyping to know their role in drug resistance.

Development of a quantitative real-time HHV-6-PCR: comparison of the results to qualitative “in-house” PCR and commercial quantitative PCR Argene CMV, HHV6,7,8 R-gene TM kit

R. Logino*, L. Mannonen, T. Karlsson, M. Lappalainen, I. Laudenschlager (Helsinki, FI)

Objectives: Human herpesvirus 6 (HHV-6) is a ubiquitous virus. Primary HHV-6 infection occurs early in childhood causing exanthema subitum. Neurological symptoms are sometimes seen. The virus may also reactivate later, especially in immunosuppressed transplant patients. The objective of this study was to develop a quantitative assay for the detection of HHV-6 genome.

Methods: The quantitative real-time HHV-6 PCR assay was developed using TaqMan chemistry and two automated sample preparation systems MagNa Pure LC and EasyMag. The assay amplifies a sequence of viral U67 gene detecting both HHV-6A and HHV-6B variants. The designed assay was compared to in house qualitative HHV-6 PCR test and to commercial quantitative Argene CMV, HHV6,7,8 R-gene kit. The clinical material of 127 whole blood specimens and 57 cerebrospinal fluid specimens, mostly from paediatric patients, were tested using these two quantitative real-time PCR methods and the qualitative PCR test in parallel.

Results: From the whole blood samples 50 were positive and 77 were negative and from cerebrospinal fluid samples 12 were positive and 45 were negative by the qualitative “in-house” HHV-6 PCR test. When the qualitative “in-house” HHV-6 PCR test was used as a “golden standard” the sensitivities of the quantitative HHV-6 PCR tests for whole blood samples were 86% for “in-house” TaqMan test and 76% for Argene’s test and specificities were 96% and 92% respectively. For cerebrospinal fluid samples the sensitivities were 92% for “in-house” TaqMan test and 80% for Argene’s test and specificities were 98% and 82% respectively. The samples that were in disagreement between the 3 tests contained very low levels of HHV-6 DNA. The reproducibility of the “in-house” TaqMan test (including DNA isolation) was good: CV% 18 (n=5, mean value 10800 copies/ml) and comparable to that of Argene’s test: CV% 18 (n=5, mean value 10800 copies/ml). The correlation of viral loads between two quantitative tests was good (R=0.95).

Conclusions: The “in-house” quantitative HHV-6 real-time TaqMan assay correlated well with the in house qualitative PCR test and commercial quantitative Argene CMV, HHV6,7,8 R-gene test. The newly developed test can be used for the diagnosis of HHV-6 infection in whole blood and cerebrospinal fluid, as well as monitoring of viral load in whole blood.

Quantification and typing of herpes simplex virus type 1 and 2 in cerebrospinal fluid by Taqman and Sybergreen real-time PCR assays for HSV encephalitis diagnosis

A. Pivert, H. Le Guillou-Guillemette, A. Ducancelle, F. Lunel-Fabiani* (Angers, FR)

Objectives: Different methods are currently available to detect Herpes Simplex Virus (HSV) in various clinical samples (cerebrospinal fluid (CSF), mucocutaneous samples). The real time PCR is now considered as a reference for the diagnosis of encephalitis, due to the poor sensitivity of the viral detection by the culture of CSF. Our objective was to provide a useful strategy for the diagnosis of HSV encephalitis in a routine laboratory. These assays allow quantification and genotyping of the viral strain.

Methods: Two new real time PCR based-assays were developed to quantify and genotype the HSV DNA in CSF samples. The first assay was based on Taqman® technology with primers and probe defined in the polymerase HSV gene. We used as quantified standard a plasmid containing the target sequence within the HSV polymerase gene. This standard was used to generate a standard curve. A PCR targeting the GAPDH gene was simultaneously performed and used as an internal control. HSV strain was retrospectively genotyped with an original real time PCR assay using SYBR®-Green technology (samples conserved at −20°C). Two pair of primers allowing the amplification of a 142pb (HSV1) and a 82pb (HSV2) targeting two adjoining regions in the polymerase gene were used. The perfect complementarity in the 3′ end of the primer and the template was needed to initiate the transcription. Discrimination of the two HSV genotypes was finally performed by the analysis of the melting curves: each PCR product provided a specific melting temperature (84.75°C and 84.75°C respectively). To assess these methods, we screened 708 CSF samples collected in patients with a suspicion of HSV encephalitis, between 2003 and 2007.

Results: Among them, we detected and/or quantified HSV in 20 samples (3%) from 12 patients. The mean viral load, calculated with the first positive sample of each patient, was 5.66 log copies/ml (2.65 to 10 log copies/ml). Genotyping PCR was performed in 11 patients: 7 encephalitis were caused by HSV 1 and 2 by HSV 2. Two patients’ samples were not detectable with this assay: a problem of sample conservation was suspected. CSF sample of the last positive patient was no longer available.

Conclusion: Our assays allowed rapid quantification and genotyping of the circulating HSV if present in CSF. These parameters may be
Interest of the PCR in the diagnosis of herpes simplex virus oesophagitis

N. Leceque*, E. Frobert, R. Guedec, A. Lukaszewicz, H. Brix, Benmansour, L. Andréleoti, V. Brodard, M.D. Diebold (Reims, Lyon, FR)

Objective: Type 1 Herpes Simplex virus is the second infectious aetiological cause of oesophagitis after Candida albicans. HSV1 oesophagitis generally occurs in immunocompromised patient causing odynophagia, retrosternal pain or dysphagia. Its current diagnosis consists in the histological examination of oesophageal lesions sampled during the endoscopy. The potential advantage of the HSV1 specific PCR assay in the diagnosis strategy of the herpetic oesophagitis remains to be assessed.

Patients and Methods: Nineteen biopsies demonstrating an evocative histological aspect of herpetic oesophagitis (presence of plurinuclear squamous cells with vitreous aspect of the nuclei) confirmed by specific immunohistochemical assay using an anti-HSV1 antibody (Dako®) and seventeen biopsies of oesophagitis without any histological proof of herpetic infection were retrospectively selected. After the dewaxing phase, DNA was extracted using DNA Blood kit (Qiagen®) and was then quantified using a spectrophotometer. A qualitative GAPDH DNA PCR was performed in order to check the good quality of the DNA extracts and the absence of PCR inhibitors before testing the samples by “Herpes consensu” kit (Argène®) for a qualitative detection of HSV DNA. For each HSV1 positive sample, HSV1 viral load levels were measured by in-house real time PCR system and expressed by the results were expressed as the number of HSV DNA copies/µg of extracted DNA (Frobert and AI, Antiviral Res., 2008).

Results: Eighteen of the nineteen HSV1 oesophagitis histologically proven were confirmed by the HSV1 specific PCR displaying a number of genomic DNA copies ranging from 1 to 3.42×10⁶ per µg of extracted DNA. Two negative biopsies by histological examination were tested positive by PCR. Their measured viral loads were respectively of 197 and 146 copies/µg of extracted DNA.

Conclusion: These preliminary data indicated a good correlation between the histological and the virological molecular diagnosis of HSV1-induced oesophagitis. The quantification of HSV1-DNA in oesophageal biopsies with a histological positive diagnosis may allow establishing a viral load threshold, estimated here at 1000 copies/ml, beyond which an aetiological diagnosis of herpetic oesophagitis could be performed even in the absence of histological evidence. However, the threshold of HSV-DNA load levels remains to be assessed in further larger prospective studies.

Evaluation of real-time PCR in the detection and management of herpes viruses infections in ulcerative colitis and oesophagitis

M. Pape*, K. Mandraveli, S. Dionysopoulou, D. Papadopoulou, O. Giouleme, A. Gatzaron, F. Frantziou (Thessaloniki, GR)

Objective: The aim of this study was to evaluate the advantages of Real-time PCR for early detection and management of virus infection of the herpes group (CMV/EBV/HSV) in ulcerative colitis (UC) and oesophagitis (ES).

Methods: 10 patients were included in this study: 8 patients, (7 men/1 woman) with UC (treated with steroids) and 2 patients with ES (1 man with a pre-existing history of ES/1 woman with no previous history of upper gastrointestinal complaints) admitted to the gastroenterology clinics of AHEPA University Hospital. Endoscopic examination was performed either at the time of admission or during follow up. Biopsy specimens obtained from the identified ulcers were submitted for histological examination for observation of viral inclusions (VI) and processed with Real-time PCR to detect CMV/HSV (arts LC, QIAGEN) and EBV (LC EBV, Roche) viral load. Whole blood specimens were processed with Real-time PCR for the referring viruses and sera were tested for anti-CMV/EBV/HSV IgG/IgM antibodies using the ELISA assay.

Results: Serological tests excluded primary infection or reactivation. In blood specimens no CMV, EBV or HSV viral load was detected. CMV and EBV DNA was detected only in tissue samples (5/8 UC, 2/2 ES). The virus combinations in UC were: EBV [1 man – 6.9×10¹⁰ copies/ml, VI(+)], CMV [1 man – 1.2×10⁴ copies/ml, VI(–)], EBV+CMV [1 man – 3.3×10⁵ – 3.1×10⁴ copies/ml VI(–)1 man – 2.2×10⁴ – 4.9×10⁴ copies/ml VI(+)]. 1 woman 2.4×10⁴ + 1.1×10⁴ copies/ml VI(–)]. In two patients no viral load or viral inclusions were detected. In one patient, although the histological examination showed specific features of virus infection, including inclusions, hyperchomaticity and atypical mitoses no viral load was detected. The results in ES were: EBV [1 man – 2.5×10⁹ copies/ml VI(+) – 1 woman – 1.6×10⁸ copies/ml VI(–)]. The patients were started on antiviral treatment with good resolution of the symptomatology. Follow-up endoscopic examination showed a clear improvement. PCR on biopsy materials was once more performed, but no viral genome could be detected.

Conclusions: This study emphasizes the possible role of herpes viruses in the pathogenesis or in reactivation of ulcerative colitis and oesophagitis. The performance of two different diagnostic techniques (histological examination/PCR) seems to optimise the specific diagnosis and leads to an appropriate therapy.

The role of microRNAs in hantavirus infections

J. Klein*, P. Sætrom, M. Berdal (Trondheim, NO)

Members of the genus hantavirus (Family Bunyaviridae) cause severe and often fatal human diseases, as haemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in America. Furthermore, in Fennoscandinavia, Psammlula virus (PUUV) causes nephropathia epidemicica (NE), a mild form of HFRS. No approved vaccine exists and therapy of hantavirus infections is mainly supportive. Except for ribavirin, which causes adverse side-effects, no potential antiviral drug has been reported to show efficacy in animal models. Thus, the development of new therapy approaches is important. In recent years microRNAs, single stranded small RNA molecules that silence the expression of genes, have demonstrated their potential in fighting virus infections in eukaryotic cells. We use PUUV as a model to elucidate the role of microRNAs in hantavirus infections to identify potential therapeutic targets. Already before the discovery of the miRNA pathway in 2001, it has been shown that plants use RNA interference as antiviral defence. Even if miRNAs are mainly involved in the natural regulation of gene expression in vertebrates, they play although a role in antiviral defence, viral tropism, latency and virus induced oncogenesis. Methods miRNAs aberrantly expressed during viral infection.

a. Global expression analysis is performed using microarry technology and validated by real time PCR.

b. Bioinformatics tools are used to identify potential targets for aberrantly expressed miRNAs and are validated in infection assays by over-expressing or depleting miRNAs.

We will present the results of this work during the meeting.

Hantaviruses in Microtus fortis and Microtus maximowiczii in far-eastern Russia

L. Yashina, N. Zdanovskaya*, L. Ivanov, R. Slonova, G. Kompanets, J. Hay, R. Yanagihara (Koltsovo, Khabarovsk, Vladivostok, RU; Buffalo, Honolulu, US)

Background: The reed vole Microtus fortis has the highest prevalence of hantavirus antigen among rodent species in the Far East of Russia. Previous studies demonstrated two distinct hantaviruses, Khabarovsk virus (KHAV) and Vladivostok virus (VLAV), in M. fortis. Since each genotype of rodent-borne hantavirus appears to be primarily associated
with one specific rodent host, there may be another principal host of KHAV or VLAV.

Methods: *Micrurus*-associated hantaviruses circulating in Khazakovsky, Amursk and Primorsky regions of far-eastern Russia were analyzed by RT-PCR. Taxonomic identification of rodent hosts was based on phylogenetic analysis of partial cytchrome b gene.

Results: Of 17 hantavirus antigen- or antibody-positive *Micrurus* sp., partial L-, M- and S-segment sequences were detected by RT-PCR in four *M. fortis* and in four *M. maximowiczii*. Hantavirus sequences recovered from *M. maximowiczii* captured in the Amursk region and from *M. fortis* captured in the Amursk and Primorsky regions resembled KHAV and VLAV, respectively. Alignment and comparison of nucleotide sequences showed an intra-strain difference up to 14% for KHAV and up to 9% for VLAV. Phylogenetic analysis, based on partial M-segment sequences, showed that hantavirus sequences amplified from *M. maximowiczii* clustered with KHAV Mf43 and Topografov virus (TOPV) carried by Lemmus sibiricus. Also, the hantavirus detected in *M. fortis* clustered with VLAV.

Conclusions: Our data demonstrate the co-circulation of KHAV and VLAV in the Amursk region of far-eastern Russia. Also, our results indicate that *M. maximowiczii* is the reservoir host of KHAV and confirm that *M. fortis* is the natural host of VLAV.

Seewis virus: genetic diversity of a soricid-born hantavirus in Siberia, Russia

L. Yashina*, S. Abramov, V. Gutorov, T. Dupal, G. Danchinoca, V. Panov, J. Hay, R. Yanozhkara (Koltsovo, Novosibirsk, Irkutsk, RU; Buffalo, Honolulu, US)

Background: Hantavirus antigens were reported more than 20 years ago in tissues of the Eurasian common shrew (*Sorex araneus*), medium shrew (*S. caecutiens*) and pygmy shrew (*S. minutus*), captured in European and Siberian Russia. Recently, a phylogenetically distinct hantavirus, named Seewis virus (SWSV), has been identified in Sorex araneus captured in Switzerland, Hungary and Finland. The Eurasian common shrew is among the most widely dispersed small mammals species in Eurasia, spanning from Europe to Siberia.

Methods: To further clarify the geographic distribution and genetic diversity of SWSV and other hantaviruses harboured by shrews, lung tissues from 29 Sorex araneus, 11 *S. tundrensis*, six *S. minutus* and six Sorex sp., captured throughout Western and Eastern Siberia (Teletskoye Lake, Karasuk, Irkutsk and Novosibirsibirsk) in 2007–2008, were analyzed by RT-PCR. To verify the taxonomic identity of the hantavirus-infected shrews, the cytochrome b gene of mtDNA was amplified by PCR.

Results: Hantavirus L- and S-segment sequences were detected in seven *S. araneus*, two *S. tundrensis*, and one *S. daphaenodon*. Overall, the sequences appeared to be genetic variants of SWSV differing from the prototype mp70 strain from Switzerland by 16–20% at the nucleotide level and 0–2% at the amino acid level. Alignment and comparison of nucleotide and amino acid sequences showed an intra-strain difference of 1–9% and 0–8% for the L-segment and 0–8% and 2% for the S-segment, respectively. Phylogenetic analysis, based on 353- and 837-nucleotides of the L and S segments, showed geographic-specific clustering of SWSV strains. At the same time, at one of the sites SWSV strains from *S. araneus* showed two separate lineages within the SWSV group.

Conclusions: The detection of SWSV in *S. araneus*, *S. tundrensis* and *S. daphaenodon* in widely separated geographic localities in Siberia demonstrates the vast distribution of SWSV among different but closely related Sor ecology. Whether this is a consequence of cross-species virus transmission or co-divergence is unclear. Also, to what extent other sympatric shrews are infected with SWSV warrants further investigation.

One tube RT-PCR for Chikungunya and Dengue, including Dengue serotype, with bead-bound probes in a liquid array detected on a Luminex instrument

M. Inoue, T. Barkham*, L.C. Ng (Singapore, SG)

Objective: In Singapore dengue is endemic while chikungunya (CK) is emerging. Repeated imported cases led in several outbreaks and over 650 notified cases in 2008. The two illnesses are difficult to distinguish clinically. Dengue’s primary vector is *Aedes aegypti*, a mosquito species that prefers to dwell indoors. CK can be spread by *Ae. aegypti* and *Ae. albopictus*. The latter prefers to dwell outdoors so control strategies differ. To support outbreak control with directed vector control management it is desirable to look for CK in clinically compatible cases. We evaluated a multiplex RT-PCR assay that detects both viruses, and also the dengue serotype, in one tube.

Methods: RNA extracted with the EasyMag system from sera submitted for clinical PCR were retrieved from frozen storage and re-tested with the test RT-PCR system. RT-PCR in one tube was performed with the Qiaegen one step RT-PCR reagent. PCR products were detected on a Luminex instrument after hybridisation with bead bound probes specific for CK and the four Dengue serotypes. Results were compared with the singleplex Dengue SYBR green method on a LightCycler and CK (Taqman probe on a Lightcycler) RT-PCR methods. A blinded series with two sera of each serotype were prepared from cultured dengue viruses and used to assess serotype specificity.

Results: Of 65 CK samples, the test system correctly identified 63: two were reported as ‘nil’ and had ct values of 39 with the Taqman singleplex assay. Of 14 Dengue samples, the test system correctly identified 14. Of 115 ‘Nil’ samples, the test system correctly identified 113 as ‘nil’ but detected two as CK. These came from patients with clinically or laboratory defined CK. All 8 of the ‘blinded’ series were correctly serotyped to Dengue serotypes 1, 2, 3 or 4.

Conclusion: The multiplex test system's performance was equivalent to the existing singleplex methods and contributed extra information in terms of the dengue serotype data. The cost, including labour, was half that of running the two separate existing assays for the two target viruses. The run time is half a working day. In areas where both of these viruses circulate combined assays that perform as well as this system may be a sensible step forwards in routine diagnostics and reveal CK in cases when it may not have been clinically suspected.

Limited yield of multiplex PCR in adults with acute pneumonia

T. Barkham* (Singapore, SG)

Objective: Most inpatients with a pneumonic illness are cared for in an open ward and do not have the causative organism identified. Our experience with SARS, in which diagnostic PCR tests were negative in early disease, showed how isolation facilities could be quickly overwhelmed if all possible cases were isolated. In these circumstances the ability to confirm an alternative diagnosis would be helpful for guiding infection control decisions. Multiplex PCR was perceived as a potentially useful tool in this respect. In order to establish systems and to evaluate their value we introduced multiplex PCR assays for adult patients with a pneumonic illness in routine non-outbreak related care in a 1200 bed hospital.

Methods: A respiratory multiplex PCR service was made available to ‘Infection’ specialists. Samples included combined nose/throat swabs, sputa and bronchial washes. Nucleic acids were extracted with an EasyMag instrument, tested with Resplex I and II kits (Genaco, now Qiagen) with Luminex detection and reported on the same day.

Results: Of 652 samples tested over 16 months from May 2006, 312 did and 340 did not have a target detected in them. Of all 652, *Streptococcus pneumoniae* was detected in 10%, *Haemophilus influenzae* 9%, *Mycoplasma pneumoniae* 1.8%, Influenza A 13%, Influenza B 9.6%, Rhinoviruses 4.3%, Coxackie/Enteroviruses 4.3%, human Metapneumovirus 2%, Parainfluenzavirus 2%, RSV 1% and
Adenovirus 0.5%. More than one target was detected in 49 samples; 47 had mixed bacterial and viral targets detected.

**Conclusion:** Although a target was detected in 48% of samples, the potential utility of the data to direct infection control activities was much less as the presence of many of the targets was felt either to be insufficient to explain the symptoms as a sole pathogen or, especially in the case of bacteria, possibly a reflection of carriage. Existing simpler and cheaper PCR systems could detect Influenza whilst the detection of *S. pneumoniae* and *H. influenzae* with culture and urinary antigen tests are more likely to reflect real disease. The yield of multiplex PCR in the adult inpatient population studied was disappointing, leaving at least 52% without a specific diagnosis. It is hard to justify the cost in routine care. It might be of greater value in paediatric and outpatient populations and in an outbreak situation when a more heterogeneous group might present for ‘assessment of fever’.

**P558 Genetic diversity of human rabies virus isolated during 2000–2008 in Morocco**

A. Fiausi*, L. Anga, H. Amarouch, J. Nourril (Casablanca, MA)

**Objective:** Rabies is an acute, progressive and fatal disease caused by an RNA virus of the Lyssavirus genus of the Rhabdoviridae family. The World Health Organization (WHO) estimates that rabies is related to the death of approximately 55,000 people per year. In Morocco human mortality due to rabies is estimated to be 20 deaths yearly. The aim of the present study was to determine the genetic diversity using sequence analysis of rabies strains isolated from humans in the Pasteur Institute of Morocco during 9 years.

**Methods:** Since 2000, 70 post-mortem brain samples from suspected rabid humans were collected from different areas of Morocco. Rabies virus was isolated from all samples and RT-PCR was used to amplify a 1300 nucleotide segment of the Nucleoprotein gene. Segment sequencing data was used for phylogenetic analysis.

**Results:** Comparisons of Nucleotide homology and phylogenetic tree analysis based on this sequence indicated that all the rabies virus isolates from Morocco belonged to genotype 1. Our result demonstrated a low diversity between different strains with high nucleotide homologies (99.1–100%).

**Conclusions:** Data presented in this study demonstrate the importance of molecular protocol for diagnosis suspected rabid humans and to evaluate the genetic diversity of strains circulating in our country. This study showed that rabies virus isolates from Morocco have a close genetic relationship.

**P559 Molecular identification of viral pathogens using PCR/ESI-MS**


**Objective:** Unacceptably high mortality rates and excessive costs associated with viral infections of sterile sites can be attributed in part, to delayed pathogen identification, which is dependent on traditional and relatively insensitive culture based methods. A rapid and reliable diagnostic test that allows for accurate identification of infected patients, and informed early therapeutic interventions based on pathogen characteristics could significantly reduce morbidity, mortality and medical costs. We propose to use the PCR/ESI-MS technology for broad-based identification of viral pathogens commonly associated with viral meningitides, encephalitis, sepsis or fever of unknown origin.

**Methods:** We have developed a PCR/ESI-MS based assay to rapidly detect and identify important viral pathogens associated with sterile sites. Viruses targeted in this assay include all species from the following genera: Adenovirus, Alphavirus, Enterovirus, Flavivirus, Herpesvirus and Human Parvovirus B19. Validation studies were performed using representative viral isolates from each target group to establish assay limits of detection (LOD), sensitivity and specificity. This was followed by a retrospective study using 54 blinded clinical specimens (CSF, urine, plasma) obtained from ViraCor.

**Results:** Viral isolates were spiked into human plasma obtained from healthy volunteers to determine assay analytical performance metrics. Target LOD ranged from 15–125 copies for the different viral species spiked. Analytical sensitivities and specificities were determined using 5× LOD of target viruses and showed 100% concordance. Blinded viral specimens from ViraCor were tested using PCR/ESI-MS and detections were compared to specific agent qPCR results from ViraCor. Of the 54 samples tested, 8 were not tested at ViraCor and 4 were not targeted by the PCR/ESI/MS assay. 41 of the remaining 42 showed concordance (98% correctness of ID) between the two assays.

**Conclusion:** The PCR/ESI-MS technology is a high throughput assay system useful for rapid detection and identification of broad range of viral pathogens from a variety of clinical specimen.
Comparative study between the new VIDAS® EBV tests and Liaison for the detection of Epstein-Barr virus VCA IgM, VCA/EA IgG and EBNA IgG antibodies in human serum samples

P.Desmottes*, A. Foussadier, L. Allard (Marcy-l’Étoile, FR)

Objectives: The VIDAS EBV reagents currently under development are aimed to detect immunoglobulins against Viral Capside Antigen (VCA), Early Antigen (EA) and Epstein-Barr nuclear antigen (EBNA). The aim of the study was to evaluate the results obtained by the 3 new automated VIDAS VCA IgM, VIDAS VCA/EA IgG and VIDAS EBNA IgG comparatively to the 4 corresponding tests available on LIAISON instrument (DiaSorin). Results concordance between techniques was determined for each kit, and a comparison of the methods was carried on acute EBV infection panels.

Methods: VIDAS EBV VCA/EA IgG and EBNA IgG reagents use specific EBV peptides coated on the solid phase to capture viral antigen immunoglobulins. The peptide-EBV IgG antibody complexes are later revealed using an anti-human IgG conjugated to Alkaline Phosphatase. VIDAS VCA EBV IgM is based on immunocapture of serum VCA IgM.

Concordance study:
- 564 samples including 93 EBV primary infection (pi), 299 EBV past infection (pi) and 172 EBV negative serum (NS) were tested with both VIDAS and LIAISON VCA IgM kits.
- 338 samples including 45 EBV pi, 93 EBV PI and 200 EBV NS were tested with VIDAS VCA/EA IgG and compared to the combined results of LIAISON VCA and EA IgG kits.
- 483 samples including 64 EBV pi, 281 EBV PI and 138 EBV NS were tested with both VIDAS and LIAISON EBNA IgG kits.

The equivocal results of both methods were excluded for the concordance calculation.

Acute EBV infection panels:
- 149 samples from 24 acute EBV infection panels were tested with the 3 kits of each manufacturer.

Results: Concordance between VIDAS and LIAISON were found to be 95%, 97% and 99% for VCA IgM, VCA/EA IgG and EBNA IgG, respectively. Equivocal results rate was observed at 7.4% for LIAISON EBNA IgG, while VIDAS EBNA IgG yielded 2%, only.

Acute EBV infection panels testing showed:
- better sensitivity for VIDAS VCA IgM compared to LIAISON VCA IgM.
- VIDAS VCA/EA performed equally to LIAISON VCA and EA IgG,
- odd reactivity of LIAISON EBNA IgG in early samples of 7 EBV panels out of 24.

Conclusions: The concordance between the new VIDAS EBV reagents and the corresponding LIAISON reagents was higher than 95%, but LIAISON EBNA IgG showed a significant rate of equivocal results. Comparison of the results of the 2 methods on acute EBV infection panels highlighted better performance for VIDAS VCA IgM and EBNA IgG than the respective LIAISON reagents.

RIDA®QUICK Norovirus: a new dimension for norovirus detection

F. Apostel*, D. Dressler, H. Leidinger, R. Fischer, P. Sander (Darmstadt, DE)

Objectives: Noroviruses are commonly associated with large outbreaks in recreational or institutional settings. They are highly infectious, thus the rapid and reliable detection of the noroviruses is paramount. The current approaches for the PCR detection of the viral genome and the use of sensitive ELISA test kits still require several hours to confirm a suspicion of norovirus infection. Due to the nature of the noroviruses both screening methods require constant monitoring and development. We here describe the development of an assay format that will detect norovirus in less than 20 minutes. The RIDA®QUICK Norovirus Assay is a flow through enzyme linked immunoassay based solely on the use of virus specific but genotype cross-reactive monoclonal antibodies for the detection of noroviruses.

Methods and Results: Monoclonal antibodies raised against VLPs or against capsid protein preparations of noroviruses from various genogroups and -types are bound to a filter membrane. Stool sample dilutions are applied to the membrane and subsequently treated with conjugate, washing buffer and substrate. Results can be obtained after 15 minutes. Signal detection is accomplished by visual reading. First study results run with 113 stool samples using predefined samples form the outbreak season 2007/2008 rendered a sensitivity 86% and a specificity of 94.6% using real-time RT-PCR as reference. Reproducibility was assessed testing 5 stool samples in three independent laboratories on three consecutive days. The results showed 100% agreement. No cross-reactivity with the usual causes for gastroenteritis was observed as well as no interference with substances commonly used to treat the symptoms of gastrointestinal diseases. The overall results obtained with the RIDA®QUICK Norovirus are in excellent agreement with those obtained using the well established RIDASCREEN® Norovirus ELISA assay.

Conclusions: The RIDA®QUICK Norovirus detection assay opens a new dimension for the quick and reliable analysis of compromised specimens. Its value for a possible prevention of outbreaks in settings where timely reaction is required is certainly indisputable.

First PCR-based detection of a central nervous system infection by Toscana virus in Piedmont region (north-western Italy)

P. Ravanini*, M. Bandi, M.G. Crobu, F. Fila, E. Gobbato, A. Godino, A.M. Nicosia, V. Quaglia, O. Bargiacchi, D. Brustia, P.L. Garavelli (Novara, IT)

Objective: Toscana Virus (TOSV) is an arthropod-borne virus transmitted by sandflies (Phlebotomus species). It was firstly isolated in 1971 in central Italy, and it is a member of genus Phlebovirus (family Bunyaviridae). TOSV can cause acute aseptic meningitis and meningoencephalitis, and in some areas of central Italy it is the main cause of these pathologies. The virus is widely spread in central and southern Italy, in Spain, in southern France and in Portugal. Despite the increasing evidence of its importance, the reports of TOSV infections in northern Italy remain sporadic.

In September 2008, during a summer stay in Italy, a 61-year-old man from England was admitted to our hospital with severe headache, fever, and mental confusion. The examination of the cerebrospinal fluid (CSF) revealed a lymphocytic meningitis. To determine the possible viral cause of the meningitis, specific nucleic acid amplifications (PCR) for Herpes viruses, Entroviruses, and TOSV were performed.

Methods: Viral nucleic acids in the CSF from the patient were extracted with Extragen kit (Novangen, Italy), and nested rt-PCR for TOSV was performed using the Toscana Virus Oligomix Alert Kit (Novangen, Italy), that amplifies a 310-bp specific fragment in the S segment of TOSV genome.

Results: The PCRs for Herpes viruses and Entroviruses resulted negative, while the rt-PCR for TOSV resulted strongly positive. The antibiotic and antiviral therapy was discontinued, and the patient recovered rapidly within a few days.

Conclusions: This is the first report of a rtPCR-based diagnosis of neuroinvasive infection caused by TOSV in Piedmont region (NW Italy) (precedent reports are only serologic). Few physicians in northern Italy are aware of the potential role of TOSV to cause CNS infections, and for this reason the number of TOSV infections could be greatly underestimated. It is therefore important to include TOSV in the differential diagnosis of all cases of aseptic meningitis and meningo-encephalitis, especially during last summer months, also in northern Italy.
Impact of rapid enterovirus molecular diagnosis in the management of aseptic meningitis in children

Introduction: Enteroviruses (EV) are the main aetiological agents of aseptic meningitis. EV may cause up to 90% of aseptic meningitis cases for which an aetiology is identified.

Objectives: The aim of the study was to evaluate the impact of EV-PCR testing on diagnosis and clinical management of children with suspiscious of aseptic meningitis.

Methods: PCR had been performed in 165 children of children with non-specific febrile illness by a commercially available reverse transcription based PCR (GeneXpert Dx system (Cepheid, Sunnyvale, CA) according to the manufacturer’s instructions.

Clinical, laboratory data and initial treatment were recorded for all patients. The turnaround time of tests and the length of hospital stay were analyzed.

Results: 165 patients with fever syndrome and/or suspected aseptic meningitis were attended in emergency room of a children’s hospital, alone 2008. RT-PCR was performed in all of them. Forty five (27.8%) had a CSF EV-PCR positive result. Fourteen (26.84%) of CSF samples had no pleocytosis. All of them were patients <3.6 months (median 1.27 months; range: 0–3.6 months). The turnaround time of tests for RT-PCR ranged from 0.16 to 2 days (mean time; 1.2 days). EV-negative patients received intravenous antibiotics in 97 cases (81.01%) while only 26 patients (57.57%) with EV-positive patients received antibiotic therapy. A positive EV-PCR result was associated with more rapid hospital discharge (median EV-PCR-to-discharge time; 3.05 days) compared with a negative result (median EV-PCR-to-discharge time; 4.44 days).

Conclusion: Rapid reporting of PCR results have a significant impact in the management and treatment of patients with EV meningitis.

Efficacy of a direct immunofluorescence assay versus shell vial culture in the detection of herpesvirus 1 and 2, and varicellazoster in skin infections
J. Reina*, V. Plascencia (Palma de Mallorca, ES)

Objectives: To prospectively evaluate the efficacy of a direct immunofluorescence assay (DFA) versus the shell-vial culture in the detection of herpesvirus 1 and 2 (HSV-1 and HSV-2) and varicellazoster virus (VZV) in skin infections.

Methods: The different skin samples were send to the virology laboratory in a virus liquid transport medium (MTV, Vircell). For performance of the DFA, 200µl per slide, were cytocentrifuged (Cytospin 3, Shandon Scientific, England) on 3 slides at 700 rpm for 10 min. After air drying the slides were fixed with acetone at −20°C for 10 min, and then stained with fluorescein-labeled mouse monoclonal antibodies to HSV-1 and HSV-2 (Syva MicroTrack HSV1/HSV2, USA) and to VZV following manufacturer’s instructions. The sample was considered adequate for the DFA if the total number of epithelial cells present was >25 per slide. A sample was considered positive if at least 2 epithelial cells with specific fluorescence were detected. The samples were inoculated into shell-vials of the Vero and MRC-5 cell lines (Vircell, Granada, Spain). The vials were incubated at 36°C and stained with the same monoclonal antibodies used in the DFA.

Results: In the 2000-2008 study period we analyzed 468 skin samples. 372 (79.4%) were considered adequate. Of them the DFA was positive in 136 (36.7%) samples and the shell-vial culture in 160 (28.4%) samples. The DFA detected the HSV-1 in 65 (17.4%) samples, the HSV-2 in 29 (7.7%), and the VZV in 42 (11.2%). The shell-vial culture was positive for HSV-1 in 57 (15.3%) samples, for HSV-2 in 21 (5.6%), and for VZV in 28 (7.5%). Using the DFA as a reference method, the shell-vial culture has an overall sensitivility of 77.9% for the HSV-1, 87.6% for the HSV-2, and 66.6% for VZV. In the 205 skin samples with a clinical suspicion of HSV-1 infection, 65 (31.7%) were positive, with suspicion of HSV-2 infection, 29 (23.3%), and with the suspicion of VZV infection, 42 (31.8%). We not isolated in the shell-vial culture a herpesvirus not previously detected in the DFA. The turnaround time for the herpesvirus isolation in the shell-vial culture was 1.7 days, and for the VZV of 4.5 days. The turnaround time for the DFA was 2.7 hours.

Conclusion: The DFA is a sensitive, rapid and easy alternative to the shell-vial culture in the detection of herpesvirus in skin samples. The shell-vial culture could be the reference method yet, but the molecular techniques have more sensitivity and specificity than the cell culture.

Epidemiological and virological study of aseptic meningitis caused by enterovirus in children

Introduction: Enteroviruses (EV) are the main aetiological agents of aseptic meningitis. EV may cause up to 90% of aseptic meningitis cases for which an aetiology is identified.

Objectives: To evaluate incidence, clinical characteristics and management of children with aseptic meningitis caused by enterovirus.

Methods: A retrospective study was conducted to determine the epidemiological, clinical, and laboratory characteristics of our patients with aseptic meningitis. The microbiological diagnoses was performed by RT-PCR (GeneXpert Dx system (Cepheid, Sunnyvale, CA) and/or viral culture by shell vial method, in CSF.

Results: 2005 CSF samples, received between January 2004 and December 2008, which corresponded to patients with a fever syndrome and/or clinical suspicion of meningitis. 134 CSF (6.68%) were found positive for EV by a RT-PCR or viral culture. A total of 131 children, median age 2.4 years, were evaluated. The study population included children <1 month to 14 years of age and was divided in 4 age groups: <1 year (64.7%), 1–5 years (16.5%), 6–12 years (16.5%), >12 years (2.3%). Most cases occurred during summer (48%) and autumn (16%). The most frequent symptoms were fever (58%), headache (19%), vomiting (19%) and neck stiffness (16.7%). The mean CSF cell count was 78/mm3, and polymorphonuclear cells were predominant in 52.3% of the cases. 28 (21.3%) of CSF samples had no pleocytosis. EV RNA was detected in 57 of 131 (43.5%) samples and EV were isolated in the culture by the shell vial method in 74 (56.5%) of children studied. Detection times of culture by the shell vial method ranged from 3 to 5 days (mean time, 3.1 days) and from 0.16 to 2 days for RT-PCR (mean time, 1.2 days).

Conclusion: PCR reduce the detection times and length of hospitalisation and plays an important role in the diagnosis and management of children with aseptic meningitis. Rapid detection and characterisation of EV meningitis is essential in making decisions for patient management and treatment.

Multi-centre evaluation of the CMV IgG assay on the Family of Access immunoassay systems from Beckman Coulter
L. Grangeot-Keros, N. Gaidot, M. Rawlins, O. Flecheux, J. Ritchie, C. Dartre, F. Bouniort*, M. Olson (Clamart, FR; Salt Lake City, UT; Marnes-la-coquette, FR; Atlanta, US; Marseille, FR; Nyon, CH; Chaska, US)

Background: The human cytomegalovirus (CMV) is a member of the Herpesviridae family. CMV is transmitted person-to-person via close non-sexual contact, sexual activity, breastfeeding, blood transfusions, and organ transplantation. CMV infection is a serious concern for women of child-bearing age, because it is a leading cause of hearing and vision loss, as well as mental retardation among congenitally-infected children. Serological confirmation of antibodies to CMV is indicative of exposure to the virus and is the principle means of diagnosis and follow-up. The Access® CMV IgG assay is a two-step immunoenzymatic (“sandwich”) assay based on paramagnetic particle, solid phase technology and chemiluminescent signal detection.
Objectives and Methodology: Access CMV IgG assay reproducibility was evaluated at three centres, using a panel of eight samples with varying degrees of reactivity and two controls. Five replicates of each sample were analyzed each day for seven days. Concordance (percent agreement) with the bioMerieux VIDAS™ and the Abbott AxSYM™ assays was evaluated at one site. Two hundred and fifty-eight de-identified residual non-selected samples were analyzed from pregnant women; mean age 21 years, range 5−65. Concordance (percent agreement) with the bioMerieux VIDAS™ and the Abbott AxSYM™ assays was evaluated at one site. Two hundred and fifty-eight de-identified residual non-selected samples were analyzed from pregnant women; mean age 21 years, range 5−65. Concordance (percent agreement) with the bioMerieux VIDAS™ and the Abbott AxSYM™ assays was evaluated at one site. Two hundred and fifty-eight de-identified residual non-selected samples were analyzed from pregnant women; mean age 21 years, range 5−65. Concordance (percent agreement) with the bioMerieux VIDAS™ and the Abbott AxSYM™ assays was evaluated at one site. Two hundred and fifty-eight de-identified residual non-selected samples were analyzed from pregnant women; mean age 21 years, range 5−65. Concordance (percent agreement) with the bioMerieux VIDAS™ and the Abbott AxSYM™ assays was evaluated at one site. Two hundred and fifty-eight de-identified residual non-selected samples were analyzed from pregnant women; mean age 21 years, range 5−65. Concordance (percent agreement) with the bioMerieux VIDAS™ and the Abbott AxSYM™ assays was evaluated at one site. Two hundred and fifty-eight de-identified residual non-selected samples were analyzed from pregnant women; mean age 21 years, range 5−65. Concordance (percent agreement) with the bioMerieux VIDAS™ and the Abbott AxSYM™ assays was evaluated at one site. Two hundred and fifty-eight de-identified residual non-selected samples were analyzed from pregnant women; mean age 21 years, range 5−65.

Conclusion: The Access CMV IgG assay provides excellent concordance with the comparison methods. The assay can aid in the diagnosis of CMV infection and may be used to assess the serological status of pregnant women with the advantage of a rapid, automated, random-access immunoassay system.

Objectives: Toscana virus (TOSV) is the main arbovirus involved in viral meningitis within the Mediterranean basin. Infected individuals acquire the virus through the bite of a sandfly, Phlebotomus spp. The vector circulates during the summer, coinciding with the pick of incidence of TOSV meningitis, mainly localised in rural areas. TOSV has been detected in pools of sandflies, but it remains unknown if there are animal reservoirs able to maintain the virus through the cold months of the year, when the vector is not circulating. We conducted a serosurveys study of TOSV in domestic animals within Granada province (southern Spain) to evaluate the prevalence of anti-TOSV antibodies in this population.

Methods: Serum samples from the following domestic animals were processed for the investigation of anti-TOSV IgG antibodies from September 2006 to April 2007: cats, dogs and horses (provided by a veterinary laboratory in Granada city), and goats, sheep, cows and pigs (provided by the Regional Laboratory of Production and Animal Health, Santa Fe, Granada, Spain). The investigation of anti-TOSV IgG antibodies was carried out by indirect fluorescence assay (IFA), using Vero cells infected with a Spanish strain of TOSV as the antigen source, and specific antisera from each animal species.

Results: A total of 1,186 serum samples were investigated by IFA, and 429 (36.2%) were positive: 138 of 286 dogs (48.3%); 127 of 213 cats (59.6%); 9 of 14 horses (64.3%); 27 of 151 cows (17.9%); 43 of 243 goats (17.7%); 74 of 229 sheep (32.3%); and 11 of 50 pigs (22%).

Conclusion: These results show that an important percentage of the domestic animals have been infected by TOSV. Further studies are being conducted to evaluate the role of some of these animals as possible reservoirs of TOSV.
envelope protein formed in lamellar and tube structures. The results of ultrastructure study were indicated that the places of HV components synthesis had different localisation. In infection by TBEV macrophages we did not observed the similar delimitations of virus component synthesis places.

**P572** Genotype of varicella zoster virus isolated from Korean elderly patients with herpes zoster

Y.J. Choi*, K.H. Kim, M.D. Oh (Koyangshi, Seoul, KR)

**Objectives:** Herpes zoster develops via reactivation of the latent varicella zoster virus (VZV) in neuronal ganglia as host immunity declines. In Korea, the seroprevalence of VZV IgG is high about 80–95%, and there is a high probability for immunosuppressed patients to develop herpes zoster. As the elderly population increases and the number of immunosuppressed patient increases, we also expect increases in herpes zoster cases. The objective of this study was to evaluate the infection rate of the VZV and to evaluate the lifetime prevalence of herpes zoster. Also, to isolate the varicella zoster virus and to determine the genotype of the VZV isolated from elderly patients in Korea.

**Method:** Serum IgG antibody titer were measured in 399 patients visiting National Cancer Center for diagnostic checkups. Lifetime prevalence of herpes zoster was evaluated through a survey on history of herpes zoster with 2054 participants visiting National Cancer Center for diagnostic checkups. VZV was isolated by cell culture technique using MRC-5 cells. To determine the genotype, ORF 22, 38, 54, 62 were amplified by PCR, and after digestion of the PCR products with enzymes pstI, bglI and small, restriction-fragment-length-polymorphism was analysed. The amplified ORF 22 PCR product were sequenced and checked for single nucleotide polymorphisms. For the determination of VZV genotype, genotype classification by Loparev was used.

**Results:** The overall seroprevalence of VZV IgG in adults was 93.9% (375/399); 91.4% (85/93) for ages 30–39, 92.5% (98/106) for ages 40–49, 97% (97/100) for ages 50–59 and 95% (95/100) for ages 60–69. The overall lifetime prevalence of herpes zoster was 13.7% (282/2054).

Of the patients with herpes zoster, 17.7% (50/282) of patients experienced postherpetic neuralgia for more than 1 month duration. The genotype of the isolates of VZV were all of J genotype; 21 (95.4%) isolates were all pstI+, bglI+, small- and 1 (4.5%) isolate was pstI– bglI+ small– (pOka) genotype.

**Conclusion:** The seroprevalence of VZV IgG ntitbody was high and it was 93.9% in adults. The lifetime prevalence of herpes zoster was 13.7%. The genotype of VZV isolated from adults over 60 years old were all of J genotype.

**P573** Prevalence of human papillomavirus types in women with normal cytology, atypical squamous cells of unknown significance and cervical intraepithelial neoplasia 1 in Madrid, Spain


**Objectives:** The presence of certain high-risk human papillomavirus (HR-HPV) types is related with higher rates of persistence of infection in cervix and with more severe lesions leading to carcinogenesis. Our objective was to study the prevalence of HR-HPV types in women with normal cytology, atypical squamous cells of unknown significance (ASCUS), cervical intraepithelial neoplasia (CIN)-1, and their geographical distribution in order to monitor all women with HR-HPV to prevent cervical carcinoma.

**Methods:** A total of 351 women attended at the Gynaecology Unit were studied for a period of two years (04/30/06–04/30/08). The presence of HPV was investigated in cervical samples by a hybrid capture test (DIGENE, Gaithersburg, USA). All positive HR-HPV specimens were studied by PCR (Linear Array, ROCHE DIAGNOSTICS) for genotyping. Cytology and/or cervical biopsy results were available from all patients.

**Results:** The most prevalent HR-HPV types in patients with normal cytology were HPV-16 (18.8%), HPV-31 (15.4%), HPV-51 (11.4%), HPV-52 (10.7%), HPV-53 (8.7%) and HPV-56 (10.7%). In ASCUS and CIN-1 specimens, respectively, the most prevalent HR-HPV types found were HPV-16 (35.7%, 17.6%), HPV-31 (14.3%, 16%), HPV-51 (28.6%, 17.6%), HPV-52 (14.3%, 11.7%), HPV-53 (7.1%, 17%), HPV-56 (28.6%, 12.2%). Multiple infections ranged from 50 to 62.8% in normal, ASCUS and CIN-1 specimens. However, multiple infections were not associated with more severe lesions.

**Conclusions:** HPV-16 was the most frequent type followed by HPV-31, 51, 52, 53 and 56. Epidemiology studies have demonstrated that HPV-16 was the type most frequently found in all countries. The other HR-HPV types differed in the different geographical areas. More studies are needed to observe if this pattern of prevalence is changed with the administration of vaccine.

**P574** Human herpesvirus 8 infection in central Tunisia: seroprevalence among different groups

N. Hannachi, N. Ben Fradj, J. Boukadida* (Sousse, TN)

**Objectives:** The epidemiology and modes of transmission of human herpesvirus 8 (HHV-8) in Tunisia are still unclear. The aim of this study is to evaluate seroprevalence of HHV8 infection in different population groups.

**Methods:** Sera from 220 children and adults were tested: 50 healthy children, 50 blood donors, 50 patients with multiple transfusions (22 multi-transfused thalassaemic children and 28 polytransfused adults) and 70 subjects with sexual risk of exposure (50 HIV-positive and 20 HIV-negative). Serological analysis was performed by using an immunofluorescence assay able to detect anti-latent and anti-lytic HHV8 antibodies.

**Results:** The seroprevalence of HHV8 was found to be 12 and 14% in healthy children and blood donors respectively. There was no difference between men and women or age group (p > 0.05). A significantly higher prevalence of HHV-8 infection was found among polytransfused patients: 60% in children and 53.5% in adults (p < 0.05). Rates of seropositivity were significantly higher than healthy subjects in patients with sexual risk: 54% in HIV infected patients and 65% in HIV-negative persons (p < 0.05). Among sexually exposed subjects, non association was found between HHV8 and HIV infections.

**Conclusion:** HHV8 infection is of intermediate endemicity in our region. Non-sexual transmission of HHV8 is operating in our geographical setting and saliva may be a potential source of HHV8 spreading in the general population. The lower prevalence of HHV8 than other herpesviruses in our country suggests a recent introduction of the virus or a lower transmissibility. There is an evidence of an increased risk of infection with multiple transfusions and sexual behaviour.

**P575** Variant GII.4 noroviruses in Italian children

S. Ramirez, S. De Grazi, C. Colomba, A. Casco*, P. Aiello, V. Rotolo, G.M. Giammanco (Palermo, Messina, IT)

**Objectives:** Among human noroviruses (NoVs), a few genogroup II strains of genotype 4 (GII.4) are dominant worldwide. GII.4 NoVs evolve rapidly and in 2006 two new epidemic variants have been identified. To investigate the circulation of GII.4 NoV variants in Italy a sequence analysis was performed on NoV strains obtained from children hospitalised for sporadic viral gastroenteritis in Palermo.

**Methods:** A total of 465 faecal specimens were collected from children (<5 years) hospitalised from January 2005 to December 2006. The presence of NoVs was detected by RT-PCR using primers JV12/JV13, targeting the region A of the RNA-dependent RNA-polymerase (RdRp) gene. NoV strains were genotyped by RdRp restriction fragment length polymorphism (RFLP) with XmnI, BstXI and sequence analysis of region A and of the ORF1/ORF2 junction region obtained with primer pair GIISKR/GIIFBN1–2–3. Phylogenetic analysis including isolates from the 2002–2004 surveillance was carried out using the software MEGA version 4.
Results: Viral gastroenteritis surveillance resulted in the detection of NoV strains in 20.9% of the patients admitted to hospital. RFLP and sequence analysis of the RdRp gene allowed to successfully characterise 59 NoV strains. Eighty-one % of the strains were characterised as GI.4, 14% as GIIb/Hilversum and 5% as GI.1. Phylogenetic analysis of region A and of the ORF1/ORF2 overlapping region of the GI.4 strains recovered in Palermo in the years 2002−2006 revealed the sequential emergence of four variants, GI.4 2002, 2004, 2006a and 2006b. The variant GI.4 2006a was detected in June and July, 2006, while the variant 2006b first appeared in August, 2006, becoming predominant thereafter.

Conclusion: The high detection rate of GI.4 NoVs in Italian children with gastroenteritis confirms their prominent role as human enteropathogens. At least four distinct GI.4 NoV variants appeared in Palermo in the last years and their dynamics of replacement and circulation in 2005−2006 appear to have matched the temporal pattern observed in Europe during the same period.

Symptomatic Parvovirus B19 infection in immunocompetent adults. Epidemiological, diagnostic, and clinical issues
R. Manfredi*, G. Martucci (Bologna, IT)

Introduction: Parvoviridae are part of air-, parenteral- and perinatal-transmitted ubiquitous viruses, whose associated signs and symptoms strongly depend on patient’s age and immune defence.

Methods: All cases of symptomatic Parvovirus B19 infection in otherwise healthy adults which came to our attention since spring 2006 were prospectively investigated and followed-up.

Results: In a 19-month period, 10 patients (7 females-3 males), with a mean age of 39.8 (range 27−46) years with a symptomatic Parvovirus B19 infection were recorded. Intrafamiliar exposure and occupational (health care) exposure were identified in 2 cases each. Clinical signs and symptoms included fever (100%), arthralgia (90%), followed by headache (80%), anaemia (70%), and rash. A mild-to-moderate dysfunction was predominant in childhood.

Conclusions: Parvovirus B19 infection may play a significant role also in the adult, immunocompetent subject, and the disease sometimes is not self-limited, requiring admission and or frequent outpatient interventions in a significant number of cases. The causes supporting a persistent infection in immunocompetent subjects have not been investigated to date, as well as the pathogenesis of myelosuppression and severe arthralgia. Symptomatic Parvovirus B19 infection is still an underestimated condition, and therapeutic perspectives are extremely limited.

Distribution of genotypes of human papilloma virus in women from different countries

The aim of this study was to determine the distribution of genotypes of HPV in women from different countries attending a consultation as part of a STI control program and screening of HPV infection.

Patients and Methods: Between 2000 and 2007 are being studied 2067 samples brushed endocervical of 1201 women (average age 28.9±7.8 years, range 18−75) from: Brazil 472 (817 samples), 360 rest of Latin America (648 samples), 248 were born in Spain (382 samples), 67 from Eastern Europe (128 samples) and 54 sub-Saharan Africa (92 samples). HPV detection was performed using a PCR fragments compared to L1 and E6/E7. The positive samples were characterised using hybridisation with probes labeled with 32P for 6/11/16/18/31/33/45/58 genotypes.

Results: HPV was found in 148 (31.3%) women in Brazil, 96 (27%) of Latin America, in 75 (30%) of Spain, 20 (28.9%) from Eastern Europe and 11 (20.4%) from Africa.

The distribution of genotypes (in percentage) is found in the table. The percentage of vaccine genotypes (HR and BR) ranged between 42 and 73%. The HPV 16 and 18 ranged between 40 and 52% of all high-risk genotypes (Africa not included).

Conclusions:
− The prevalence of HPV infection was similar in all groups.
− The 16 genotype was distributed equally in all countries included in this study.
− The 18 genotype was less common in Spanish women.
− The genotype was detected in only 45 women in Latin America.
− The high-risk genotypes vaccine account for half of oncogenic genotypes detected.
Seroepidemiology of Varicella zoster virus infections in Greek adults during a two-year period


Introduction: Varicella zoster virus (VZV) is a human alphaherpes virus, which causes varicella (chickenpox) and herpes zoster (HZ, shingles). Varicella is a common illness of early childhood that occurs during primary infection. Zoster usually occurs in adults or immunocompromised patients. It is caused by reactivation of the virus in latency after the primary infection in cells of the dorsal root or cranial nerve sensory ganglia.

Objectives: The aim of this study was to determine the current status of VZV immunity and to estimate the incidence of varicella and HZ in hospitalised adults over the years 2007–2008.

Methods: A total of 918 serum samples from adult patients (17–65 years old), hospitalised in several clinics of the General Hospital “G. Gennimatas”, were examined during a two year period. The sera were tested for specific IgG, IgM and IgA class antibodies by an enzyme-linked immunosorbent assay (VIR-ELISA-ALPHAIDIA-BELGIUM).

Results: The seroprevalence for IgG, IgA and IgM was 81.5% (748/918), 2.17% (20/918) and 0.98% (9/918) respectively. The susceptibility rate for VZV was found to be high, 18.5% (270/918). VZV infection was clinically confirmed in 19 patients (2.9%, 19/918). The incidence of HZ and HZ2 infections occurred in a small number of adults (2.09%), the risk of primary infection remains high, due to the high sensitivity rate (18.5%) to the virus.

Clinical and genetic analysis of human bocavirus in children with lower respiratory tract infection in Taiwan

P.267


Objectives: Human bocavirus (HBoV) has a potential role in the development of acute respiratory disease in children. Recently, the prevalence of this virus has been studied worldwide. We conducted the first clinical and molecular study at the Centers for Diseases Control, Taiwan, to investigate the genomic and epidemiological profiles of HBoV infection in Taiwan.

Methods: Throat swabs or nasopharyngeal aspirates were obtained from paediatric hospitalised patients with acute lower respiratory tract infection. Specimens that were negative for other respiratory viruses by molecular screening were examined for HBoV. Complete viral genome was amplified and sequenced to re-construct their phylogenetic trees.

Results: HBoV was detected in 30 (6.5%) of 531, of these positive cases 56.67% at the age less than 2 years old. Sequences comparison showed highly conserved and similarity with Taiwan HBoV among different isolates with 2 groups of HBoV co-circulated. There is no genotypic difference between the strains from Taiwan and other countries. Split decomposition tree and BootScan showed a possible recombination of different HBoV strains.

Conclusions: HBoV might have circulated in Taiwan for a certain period and is involved in the aetiologic agents responsible for lower respiratory tract infection in children. Evolutionary networks suggest that HBoV might have an opportunity for interbreeding of virus and genetic recombination among the different genotypes.

Activation of interferon response in human PBMC by avian influenza H5N1 virus

E. Lalle*, C. Castilletti, L. Bordi, R. Chiuppi, M.R. Capobianchi (Rome, IT)

Objectives: The severe complications of HPAI H5N1 strongly suggest a key role for over-exuberant immune response pathogenesis. Higher plasma levels of chemokines and proinflammatory cytokines have been observed in patients with H5N1 virus infection.

The aim of our study was to compare the capability of H5N1 and H3N2 viruses to induce IFN-α and -γ and to activate the IFN stimulated genes in human PBMC from healthy donors. Moreover we analysed the differential modulation of a broad range of TLRs pathway’s genes.

Methods: Normal PBMC were exposed to both viruses at different MOI. IFN-α and -γ induction was measured both at mRNA level and cytokines release; IFN stimulated genes (PKR, MxA, 2′-5′ OAS) were also analysed. The profile of expression of TLR signalling pathway related genes was studied by a Real-Time PCR array.

Results: The amount of H5N1 RNA was significantly lower than that of H3N2 after 1 hour of adsorption. The results indicate that H5N1 is able to induce more efficiently IFN-α and γ mRNA as compared to H3N2. Interestingly, released IFN was observed at 3 hours only in PBMC induced by H5N1, and not by H3N2. The induction of a set of genes involved in the innate immune response, was detected at 3 h.p.i. in PBMC exposed to H5N1, and not to H3N2, however, at later times, the extent of activation of these genes was similar. TLR array analysis suggests a different mechanism of INF induction for H5N1 and H3N2

Conclusion: HPAI H5N1 is able to stimulate, in a dose- and time-dependent manner a coordinate induction of IFN-α and -γ in normal PBMC. Type I and II IFN response to H5N1 is more rapid and intense as compared to H3N2. A more rapid response is also observed for IFN-activated genes, but the overall extent is not different from H3N2. The different kinetics are not accounted for by differences in cell bound viral RNA. The IFN induction mechanisms may be different from those based on interaction of viral RNA with TLR or with other cellular receptors. Different kinetics of IFN response may have pathogenic significance.

Protein C, protein S levels and other haematologic parameters in patients with Crimean-Congo haemorrhagic fever

Z. Özkurt*, S. Erol, A. Kadaml, K. Özden (Erzurum, TR)

Objectives: Crimean Congo haemorrhagic fever (CCHF) may be fatal viral haemorrhagic fever. Massive bleeding is the most frequent cause of death in patients with CCHF. Coagulopathy and DIC is the main cause of bleeding in CCHF and other viral haemorrhagic fevers. This study is conducted with prospectively to investigate which parameters disordered in coagulation cascade.

Methods: Fifty CCHF patients hospitalised at Ataturk University Medical School, Department of Clinical Bacteriology and Infectious Diseases enrolled the study. Protein C, Protein S, Factor VIII, PT, aPTT, INR, D-dimer, fibrinogen levels were investigated in these patients.

Results: Some pathologic changes were detected in levels of measured parameters. The most important changes were detected D-dimer values, and the values were increased in 48 (96%) of patients. Second important changes were detected Protein S values; there were protein S deficiency in 27 (54%) patients with CCHF. Both protein C and Protein S deficiency were found 8 (16%) patients. In all patients have protein C deficiency, protein S levels also decreased. Decreased protein C and S levels at admission improved (returned to normal) later period of the diseases. Results are shown in Table 1.

Table 1. Haematologic parameters in patients with Crimean-Congo Haemorrhagic fever

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n, %</th>
<th>Protein C</th>
<th>Protein S</th>
<th>Factor VIII</th>
<th>D-dimer</th>
<th>PT</th>
<th>aPTT</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, n (%)</td>
<td>50</td>
<td>42 (84)</td>
<td>23 (46)</td>
<td>48 (96)</td>
<td>40 (80)</td>
<td>2 (4)</td>
<td>40 (80)</td>
<td>39 (78)</td>
</tr>
<tr>
<td>High/Prothromb., n (%)</td>
<td>2 (4)</td>
<td>40 (80)</td>
<td>8 (16)</td>
<td>11 (22)</td>
<td>10 (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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*P-values for other comparisons were not reported.
Conclusions: Protein C and protein S consumption may be increased and/or synthesis may be decreased in CCHF. Pathologic changes in these haematologic parameters and others should be investigated to explain the pathogenesis of the disease and therapy.

**Analysis of the importance of nuclear export of the influenza A virus NS1 protein for viral replication**

J. Tynell*, K. Melen, J. Julkunen (Helsinki, FI)

Objectives: The NS1 protein of influenza A virus is a multifunctional protein responsible for the inhibition of host cell immune responses. Through its action in the nucleus and the cytoplasm NS1 functions to shut down host cell interferon production and to limit the effects of interferon-inducible antiviral proteins. To study the importance of the intracellular localisation of NS1 we created a recombinant influenza A virus of the strain A/Udorn/72 bearing a mutation to inactivate the nuclear export signal (NES) of NS1.

Methods: The recombinant virus was created using a 12 plasmid transfection system. The intracellular localisation of NS1 was visualised by immunofluorescence microscopy. The growth properties of the virus were analyzed by measuring its growth kinetics on MDCK cells at different multiplicity of infection (MOI) values. The cytotoxic production of infected A549 cells was measured by ELISA.

Results: of infected A549 cells was measured by ELISA.

The mutated NS1 localised and remained in the nucleus even at late stages of infection. The recombinant virus replicated to titers 10-fold lower compared to the wild type virus and was not able to inhibit the production of interferon-alpha or interferon-beta by the host cell.

Conclusion: The nuclear export of NS1 appears to be important for the replication of influenza A virus and for its ability to inhibit host cell interferon production, although the exact cytoplasmic role of NS1 responsible for the observation cannot be elucidated from these results. The possibility of other properties of NS1 besides the NES being affected by the induced mutation also cannot be ruled out.

**Prevalence of human papillomavirus types in women with cervical intraepithelial neoplasia in Madrid, Spain**


Objectives: Infection with high-risk human papillomavirus (HR-HPV) types is the main cause of cervical cancer. Prior to vaccination, it is important to define the prevalence of HR-HPV in each geographic area. Our objective was to study the prevalence of HR-HPV types in our country in patients with CIN2+ results in cervical samples and investigate the relationship between HPV types and histological data to ascertain whether HPV types differ geographically.

Methods: A total of 458 women attended at the Gynaecology Unit at our hospital for a period of two years (04/30/06-04/30/08). The presence of HPV was investigated in cervical samples by a hybrid capture test (DIGENE, Gaithersburg, USA). All positive HR-HPV specimens were studied by PCR (Linear Array, ROCHE DIAGNOSTICS) for genotyping. Cytology and/or cervical biopsy results were available from all patients.

Results: This study included 149 women with normal cytology, 14 atypical squamous cells of unknown significance (ASCUS), 188 cervical intraepithelial neoplasia (CIN)-1, 57 CIN-2, 34 CIN-3 and 16 with cancer. The most prevalent HPV types in CIN2+ were HPV-16, 18, 31, 33, 51, 52, 56 and 58. HPV-16 was implicated in CIN-2 (56.1%), in CIN-3 (47.1%) and in cervical carcinoma in situ (CIS) (87.5%). Multiple infections in CIS specimens, comprising more than 2 HPV types, were found in 31.3% of patients. HPV-16 was present in all multiple infections. However, the presence of more than 1 HR-HPV type was not associated with an increased risk of high grade lesions.

Conclusions: HPV-16 was the most common type in patients with CIN2+ results. Consistent with other authors, also in Spain, HPV-16 and HPV-18 were the two most common types found in CIN2+ lesions.

Interestingly, in our study HPV-18 caused only 7.5% of CIN2+, in contrast with the fraction attributable to HPV-16 (58%). In our study, the prevalence of HPV-16 in CIS was higher (87.5%) than in other studies performed in other countries (50−55%). Regional variation in the distribution of HR-HPV types should be considered to design screening tests and vaccination programs.

**Tuberculosis: epidemiology and clinical disease**


Objective: Tuberculous meningitis (TM) as a marker of uncontrolled tuberculosis in population is still a health problem in developing countries. The aim of this study is to determine epidemiologic, diagnostic, clinical features and outcome of TM in adults who were admitted in Cukurova University Hospital.

Methods: Thirty-eight patients treated in Infectious Diseases Department between January 1995 and December 2004 were investigated retrospectively.

Results: Of patients, 20 were male (52.6), and 18 (47.4) were female. Mean age of the cases was 29 (min 15, max 60, SD ±13). Diagnosis was confirmed by microbiological evidence (culture, polymerase chain reaction or acid fast stain positivity) in 15 cases (39.5%), by histopathological, radiological and clinical findings in 13 (34.2%), and through response to therapy in 10 (26.3%) of patients. Mycobacterium tuberculosis (MT) was recovered from clinical samples in 10 patients (26.3%), whereas acid fast stain positive only in 4 (10.5%) patients. Drug resistance were detected for 2 isolates from CSF (One resistant to both isoniazid and rifampicin, and another resistant to both ethambutol and rifampicin). Both patients who has resistant MT were recovered with sequela. The initial examination of cerebrospinal fluid (CSF) showed cell counts ranging from 10 to 1530 with mean cell count 263/mm³, mean protein level 227 mg/dl (min 16, max 700), mean lactate 6.4 mmol/L (min 2.2-max 11.3). Three (7.9%) of the patients were classified as stage I, 15 (39.5%) as stage II, and 20 (52.6%) as stage III according to their clinical findings. Overall, 5 (13.2%) of the patients had a full recovery, 25 (56.7%) had recovery with sequelae and 8 (21.1%) fatal cases were observed. All deaths were associated with stage III. No patient had HIV infection and in 33 (86.8%) of the patients there were no evidence of underlying pathology. Three patient had diabetes melitis, one has Crohn disease and one was pregnant. All of the patients received four major antituberculous drug regimen for 3−4 months and then INH and RMP plus steroid around 4 weeks, except ones with resistant MT. The mean duration of therapy was 12 months. Hydrocephalus was evident in 16 patient (42.1%), and 5 (13.2%) of them required ventriculo-peritoneal shunt.

Conclusion: TM is still an important health problem with high mortality and cost and severe sequela even in adults in developing countries. Clinicians must be aware and administer promp terapy to reduce complications.

**Genitourinary tuberculosis: a 12-year experience**

D. Stasio, A. Skolarikos, E.M. Fakirit, G. Alevisatos, M.A. Ntrivala, C. Deliceliotis, E. Papafragans (Athens, Marousi, GR)

Background: We present our experience with genitourinary tuberculosis (GUTB).

Methods: We reviewed the medical records of patients who have been treated for GUTB. The epidemiology, clinical presentation, diagnosis, treatment and long-term follow-up are presented.

Results: 27 males and 8 females were treated for GUTB in our hospital. 19 patients were admitted for pulmonary infection, 10 patients for upper tract dilation and LUTS, 4 patients for fever of unknown origin, one patient with painful testicle and one patient for haematuria. Urine culture in liquid media (Mycobacteria Growth Indicator Tube; MGIT) was
positive in 27 patients at a mean time of 12.1 years, while culture in solid media (Lowenstein-Jensen medium; LJ) was positive in 23 patients at a mean time of 26 days. Acid-fast stain (Ziehl-Neelsen; ZN) was positive in only 9 patients raising the positive predictive value (PPV) of MGIT, LJ and ZN in 90%, 63% and 25%, respectively. Mono-resistance was noted in 4 isolates (isoniazid, streptomycin, pyrazinamide), poly-resistance in 2 isolates (isoniazid/streptomycin; isoniazid/pyrazinamide/ethambutol) and multi-resistance in one isolate.

Urological intervention (14 double-J stents; 2 nephrostomy tube, 1 nephrectomy) was needed in 17 patients at presentation. All patients were treated for a period of 9 months with the exception of one patient who was treated for 12 months. The mean follow-up was 44.3 months (range 5–146). During follow-up two patients died because of sepsis secondary to urinary tract manipulation. No other complication was found and the urinary tract system was normal in 24 patients at the last follow-up.

Conclusions: GUTB has a wide spectrum of clinical and radiological findings. A high clinical suspicion is warranted for diagnosis. MGIT shows the highest PPV among the diagnostic studies. The incidence of mono-resistance or multi-resistance to first-line anti-tuberculosis agents still remains relatively low. Complications secondary to the infection or its treatment are rare and most of the patients are cured following appropriate treatment.

P586 Pulmonary Mycobacterium simiae disease in Iran's national referral centre for tuberculosis

P. Baghaei Shiva* (Tehran, IR)

Background: Various types of non-tuberculosis Mycobacterium (NTM) can affect human and can cause either symptomatic or asymptomatic infection. In this particular study, we intend to determine the clinical, radiological, and treatment of M. simiae in patients in our referral centre in Iran.

Methods: This retrospective study is conducted at Masih Daneshvar Hospital, a TB referral hospital in Iran. All patients presenting to our centre between 2002–2008 with confirmed infection with M. Simiae are included in the study. For all patients, smear and culture for AFB and drug susceptibility testing were performed. Also, PCR, identification methods for NTM, and high-resolution CT scan were carried out as well. All patients with confirmed M. simiae diseases were treated according to ATS recommendations.

Results: Totally, 14 cases of M. simiae were identified in our centre. Eight were female, all but one were Iranians, and the mean age of the patients was 53±17.76 years. Just one patient was HIV positive. The most frequent symptoms were cough (100%), Fever (92.9%), weight loss (85.7%), 57% of the patients had nodular lesions. As well, each bronchiectasis or cavitation was present in 50%. All patients were resistant to all first-line drugs. 12 patients reached cure and two patients failed the treatment. There was no recurrence of the disease by any of the patients after undergoing treatment.

Conclusion: M. simiae may present with clinical and radiological manifestations consistent with tuberculosis, and be resistant to anti-TB agents. NTM such as M. simiae should be sought to shorten the period of treatment, lessen the adverse effects, and reach a more efficient treatment.

P587 Tuberculosis in the oral cavity: a systematic review of the published evidence

A. Kechaiga, O. Kakisis, M. Falagas (Manchester, Athens, GR)

Objectives: The incidence of oral tuberculosis, observed mainly secondary to pulmonary infection has been diminished to less than 1% following the successful implementation of antituberculous drug therapy. The recent outbreaks however, combined with emerging resistance in antituberculous medication warrant an increased suspicion of mycobacterial involvement for persistent or atypical lesions of the oral cavity.

Methods: We sought to review the published reports of mycobacterial infection of the oral cavity in the literature from 1950 until today and analyze the reported findings.

Results: Oral tuberculosis infection appears in all regions of the oral cavity (soft and hard palate, uvula, buccal mucosa, gingivae, lips, tongue, maxilla and mandible) more often in males than in females (age mean 37 yrs), predominantly in the form of ulcerative lesions covered with slough and elevated margins, but also as swellings, nodules, pappulae or granulomatous plaques. A large proportion of patients have had multiple prior failures to treat by conventional antibiotics and/or steroid treatment. Diagnostic investigation is important and microbiological staining and/or culture has always been the golden standard for oral Mycobacterium tuberculosis showing however varying sensitivities in oral tissues. The importance of PCR detection is manifest and discussed.

Conclusions: The investigation for a mycobacterial infection in oral cavity lesions should be intense in the dental office due to the emergence of the disease and the importance for infection control. Half of the times oral tuberculosis leads to the diagnosis of pulmonary tuberculosis with significant benefit for the patients. A conventional X-ray and biopsy is imperative, rapid microbiological identification and drug susceptibility required and awareness warranted.

P588 A new approach to tuberculous meningitis in Spain

V. Moreno*, E. Valencia, G. Ramírez-Olivença, J. González Lahoz (Madrid, ES)

Objectives: Tuberculosis (TB) is an increasing health problem in Spain due to immigrants and patients HIV+. Tuberculous meningitis (TM) is one of the most important manifestations and our objective was to analyse the characteristics and evolution of a group of Spanish and non-Spanish patients with TM.

Methods: We reviewed the clinical history of 25 patients with TM hospitalised (2002–2008). Statistical study was done by SPSS 13.0. A comparative study was performed of variables related to nationality.

Results: TB was only meningeal in 17 patients and disseminated with meningeal localisation in 8. In 10 cases there was a previous episode of TB and 20 (12 Spanish and 8 immigrants) were HIV+ (80%). Mean age was 43 years, 13 were men (52%) and 14 were Spanish (56%). The other were from Equatorial Guinea (20%), Ecuador (12%), Nigeria (8%) and Peru (4%). Fever and headache were the most frequent symptoms (96%), 17 patients (68%) had confusion and 14 were disoriented. The most important criteria for TM diagnosis were cerebrospinal fluid biochemical alterations with elevated cells (mean: 298 cells/mm3, all cases lymphocytes), elevated protein level (mean: 171 mg/dl), decreased glucose level (mean: 34 mg/dl) and elevated adenosine deaminase level (mean: 15 IU/L). Koch bacilli grew in sputum in 16%, in urine in 20% and in spinal fluid in 8%. All cases were sensible TB. 21 (84%) patients were treated with 4 drugs and 15 (60%) received dexamethasone. One year after treatment 17 patients were cured, 4 were dead (16%) and 4 left pursuit without finishing the treatment. Three subjects cured with major sequels. The use of dexamethasone did not influence evolution. Spanish patients were men most frequent than immigrants (71% vs 27%), heavy alcohol drinkers (50% vs 9%), smokers (79% vs 18%), intravenous drug users (71% vs 0%) and had more disseminated tuberculosis (43% vs 18%). None of immigrants patients had culture positive, all were treated with 4 anti-tuberculous drugs and there was no differences between clinical manifestations, presence of HIV infection, cerebrospinal fluid biochemical alterations or evolution.

Conclusions: TM is a very important health problem with an elevated number of sequels and mortality. Half of our patients were immigrants and 80% were co infected with HIV. The clinical and evolutionary characteristics of immigrants were comparable to Spanish patients but
their demographic, microbiological ant treatment characteristics were different.

**P589** Human pulmonary tuberculosis due to *Mycobacterium microtii*: description of 6 recent cases in France

S. Godreuil* (Montpellier, FR)

Objectives: *Mycobacterium microtii* belongs to *Mycobacterium tuberculosis* complex (MTBC). Although it was considered for a long time as nonpathogenic for humans, the first human tuberculosis due to *M. microtii* has been reported in 1998. Here, we report the clinical, microbiological and molecular characteristics of 6 human pulmonary tuberculosis due to *M. microtii* in France.

Methods: We carried out a retrospective study of cases diagnosed between 2000 and 2007. The isolation of *M. microtii* was performed by culture from respiratory samples and the isolates were identified as *M. microtii* by the following molecular methods: (i) GenoType Mycobacterium AS (Hain, Lifescience, Germany), (ii) a spoligotyping, and (iii) specific signature at gyrB gene determined by sequencing.

Results: Out of the 6 patients (4 males and 2 females), 5 patients (83%) were born in France and 1 patient was native of Senegal and was living in France since 1990. Three patients had underlying conditions such as HIV infection (n = 1), diabetes mellitus (n = 2). For 6 patients, any notion of tuberculosis in childhood or in the entourage has been found. No patient was exposed to pets specially cats or small rodents. The median time between the onset of clinical symptoms and the diagnosis of tuberculosis was 10.2 weeks (range, 16–72 weeks). The presenting symptoms were productive cough (6 patients), night sweats (2 patients), thoracic pain (1 patient) and there was no fever or haemoptysis. Chest radiographs revealed cavitating pulmonary lesions in 5 patients and bilateral disease in 2 patients. For the 6 patients, aephotometric examination showed acid-fast bacilli. The cultures were positive in an average time of 48 days. Spoligotyping revealed the llama spoligotype for 5 isolates and a new pattern for 1 isolate. Disseminated infection was observed in 3 patients. Two patients were treated with tri-therapy (rifampin, isoniazid, pyrazinamide), and 1 patient received only isoniazid. Treatment regimens were divided into four groups: 3 patients were treated with tri-therapy (rifampin, isoniazid, pyrazinamide), 3 with tri-therapy regimen plus ethambutol. Treatment period ranged from 6 to 12 months. The 6 patients had favourable outcomes.

Conclusion: The prevalence and clinical importance of *M. microtii* have been previously underestimated. Further studies based on molecular methods are needed to better understand the epidemiology and the transmission of this pathogen to humans.

**P590** Risk factors of paradoxical response in HIV-negative patients with peripheral lymph node tuberculosis


Objectives: The paradoxical response (PR) of lymph node tuberculosis (TB) during anti-tuberculosis therapy was well-known phenomenon in non-HIV infected patients as well as in HIV-infected patients. But data on risk factors are limited. The aim of this study is to elucidate the clinical characteristics and risk factors for PR of peripheral lymph node TB in non-HIV infected patients

Methods: The medical records were reviewed between Jan 1997 and Aug 2007 on non-HIV infected patients who were >16 years of age with peripheral lymph node TB at a 2,200-bed tertiary teaching hospital. Patients were classified as having confirmed TB if clinical symptoms were positive for *M. tuberculosis* on culture, acid-fast bacilli (AFB) stain, or *M. tuberculosis* PCR. Patients were classified as having probable TB if the histologic finding of biopsy tissue showing necrotising granuloma and successful response to anti-tuberculosis therapy. PR was defined as the clinical or radiological worsening of pre-existing TB lesions or the development of new lesions in patients who had received anti-tuberculosis therapy for at least 2 weeks.

Results: A total of 300 non-HIV-infected patients with peripheral lymph node TB were enrolled. The mean age (+standard deviation) of the patients was 37.9 (+13.9) years and 232 (77%) were female. Of these, 267 patients (89%) had cervical lymphadenitis. By clinical categories, 234 patients (78%) were classified as confirmed TB, and the remaining 66 (22%) as probable TB. Necrotising granuloma, AFB stain, TB PCR, and cultures for *M. tuberculosis* were positive in 192 of 300 (64%), 106 of 300 (35%), 156 of 188 (83%), and 42 of 141 (30%), respectively. The PR occurred in 61 (20%, 95% CI 16.2% to 25.3%) of 300 patients, the median onset time of PR was 7 weeks (interquartile range, 4–12 weeks). Thirty-one patients (53%) were closely observed with anti-TB medications only, 16 (26%) had surgical lymph node excision, 10 (16%) had fine-needle aspiration performed, and 2 (3%) received corticosteroid therapy. The young age (OR 0.96), male sex (OR 2.7), large nodes (OR 1.3), the presence of local tenderness (OR 2.9), and prior treatment of TB (OR 2.4) were independent risk factors for PR in a logistic regression analysis.

Conclusion: The PR was common, occurring in one-fifth of patients. The risk factors of the PR were young age, large nodes, the presence of local tenderness, and prior treatment of active TB in non-HIV patients with peripheral lymph node TB.

**P591** *Mycobacterium marinum* infection in a striped bass farm in northern Italy

E. Bozetta, K. Varella*, I. Giorgi, M. Fioravanti, M. Pezzolato, R. Zanoni, M. Prearo (Turin, Bologna, IT)

Objectives: Striped bass (a hybrid of Morone spp.) is an economically and recreationally important farmed fish in US. In Italy this species is not highly valued, but recently its farming is becoming more popular with the Italian consumers. Mycobacterial infection in wild and farmed Morone spp. are often reported in US and other countries, *Mycobacterium marinum* is a frequent isolate. To date, few cases of mycobacterial infections were described in fish reared for human consumption in Italy. Here we describe an episode of *M. marinum* infection in a striped bass farm located in Northern Italy.

Methods: A striped bass was reported to the fish disease laboratory following the detection of yellow-brown nodules on the gills. The microscopic identification of multiple mycobacterial granulomas suggested further investigations in the fish rearing. Forty fishes were randomly selected sacrificed and necropsied in order to investigate the presence of the infection and to identify the mycobacterial species involved. At necropsy samples of gills, liver, spleen, kidney, skin and muscle were collected for histological examination and microbiological investigations.

Results: At gross examination ulcerative cutaneous lesions, yellow muscus with red nodules on the gills, splenomegaly and miliary granulomatous lesions in liver, kidney and spleen were observed in all animals. Microscopically, granulomas presented a central eosinophilic area (necrosis) surrounded by inflammatory cells and enclosed by a thin capsule. The nodules resulted positive at Ziehl-Neelson staining, displaying a variable amount of acid-fast bacilli. From all the tissues, with the exception of the muscle, photochromogenic colonies were identified; the purified isolates were phenotypically and biochemically characterised as *M. marinum*. Paraclinical and virological investigations resulted negative.

Conclusions: This episode suggests that striped bass can represent a reservoir of mycobacterial infection in Italian rearings too. It is important to emphasize that, although granulomatous lesions could be observed in various organs, the mycobacterial infection was not responsible for mortality in the farm. Due to the low death rate observed, the risk of human exposure to the infection by manipulation and consumption of the contaminated fish belonging to this species has not to be disregarded.
Particularities of tuberculosis in teenagers horizontally infected with HIV-1 during infancy

A. Capsa*, F Dumitreasa, L. Giubelan, I. Niculescu, A. Romanescu, C. Miu, A. Rosca (Craiova, RO).

Background: Historically, Romania cumulates, worldwide, the largest number of horizontally HIV-1 infections in children. Meanwhile, the southern part of the country (Oltenia province) registers the highest incidence of tuberculosis (TB) from Europe (about 160.5/100000 inhabitants). Contextually, HIV infected teenagers have a high risk of tuberculosis – from exogenous sources or endogenous reactivation.

Objectives: To evaluate the incidence, clinical and evolutive aspects as well as immunological and virusological characteristics in teenagers having both TB and HIV-1 infection horizontally transmitted.

Methods: A longitudinal study (01.01.2002–31.12.2007); we have evaluated 415 teenagers (born 1988 and 1989), horizontally infected with HIV-1 during their infancy, under surveillance of Regional Center for Evaluation and Monitoring of HIV/AIDS Infection, Craiova, Oltenia province, Romania. TB workup was based on epidemiological, clinical, imaging (X-ray, CT scans), microbiological (smears, culture) and immunological (Quantiferon TB, PPD skin test) data.

Results: 76 (18.3%) patients (Ps), HIV infected teenagers (male/female = 41/35) have been diagnosed with TB. Exogenous sources have been identified in 9 cases (11.8%) vs 67 Ps (88.2%) having probably an endogenous reactivation of a latent infection. Clinically we have encountered: pulmonary TB – 48 cases (63.2%), non-pulmonary TB – 19 cases (25%) and mixture – 9 (11.8%) cases. 20 Ps (26.3%) had a bacteriological confirmation of tuberculosis. The average CD4 count has been 185.2±171.1 cells/mm³ while the average viral load has been 5.6±5.4 log copies/ml. Relative to antiretroviral treatment (ART): 23 Ps (30.2%) was naive before TB diagnosis (19 of them newly diagnosed as HIV infected) while 53 Ps followed ART (average 2.7 regimens/Ps, 28 of them – 36.8% – being multiexperienced). All Ps received ART, anti-bacillary treatment, pathogenic and supportive care. 21 Ps (27.7%) had an inflammatory reconstitution syndrome and 3 Ps (3.9%) recorded a complete recovery, 20 Ps (26.3%) registered relapses, 4 Ps (5.3%) had an inflammatory reconstitution syndrome and 3 Ps (3.9%) remained with sequelae. We have recorded 28 deceases (36.8%).

Conclusion: The incidence of TB among HIV infected teenagers is high in the Oltenia province; pulmonary TB is the most frequent clinical type; TB occurs regardless of the CD4 count or viral load level; evolution of TB is severe in Ps having advanced HIV infection, with multiple ART regimens, even with a proper tuberculosis treatment.

Non-tuberculous Mycobacteria: incidence and clinical significance


Objectives: There is geographic variability in the prevalence of mycobacterial species. However there have been only a few incidence data for nontuberculous mycobacteria (NTM) in Asian countries. Also clinical significance of NTM infection is still unclear. To describe the incidence and clinical significance of NTM infection in a single centre of Korea.

Methods: Between January 2004 and December 2007, we analyzed a total of 19,593 clinical samples from 10,057 patients who were requested for mycobacterial cultures. NTM species were identified by using PCR-restriction fragment length polymorphism methods (PRA).

Result: A total of 160 specimens (94 sputum, 55 bronchial washing, 5 peritoneal dialysate, 4 pus, 1 lymph node, 1 bone marrow) from 100 patients (50 male, 50 female) were culture-positive for NTM. The mean age of the patients was 60.6±12.8-years old. Among a total of 100 patients with culture-positive for NTM, 47 cases had COPD, 40 previous tuberculosis history, 10 malignancy, 9 autoimmune disease, and 3 HIV-infected. The most frequently isolated organisms were M. avium complex (n=86, 53.8%), followed by M. abscessus (n=48, 30%), M. fortuitum complex (n=9, 5.6%), M. gordonae (n=4, 2.5%), and unclassified (n =13, 8.1%). Forty-seven patients with NTM infection (43 pulmonary disease, 2 peritonitis, 2 disseminated disease) received anti-NTM treatment. Two patients were reactivated NTM after treatment. Only 49 cases (30.6%) were smear-positive for acid-fast bacilli (AFB) by microscopy.

Conclusion: The common species of NTM in our centre were different from other countries. In our centre, the incidence of NTM disease using PRA demonstrated in 47% of patients with culture-positive for NTM. The sensitivitiy of AFB-smear for NTM infection was relatively low, therefore culture for NTM should be considered in patients with suspicion of mycobacterial infection but negative AFB-smear result.

Prevalence of Mycobacterium tuberculosis drug resistance in a Spanish teaching hospital during a six-year period


Objectives: An increase in Mycobacterium tuberculosis drug resistance has been registered in the last few years in industrialised parts of the world. Rapid detection of M. tuberculosis strains resistant to antituberculosis drugs is probably the most important factor to minimise the spread of contagion. The aim of this study is to evaluate the drug resistance of culture positive cases of pulmonary and extra-pulmonary tuberculosis during a six year period in a Spanish teaching hospital.

Methods: 343 strains of Mycobacterium tuberculosis were obtained from samples, received at the Microbiology Department (Hospital Universitario de la Princesa, Madrid) from January 2003 to December 2008. Samples were collected by standard procedures. They were examined by auramine stain and inoculated on Lowenstein Jensen (LJ) media and BACTEC MGT 960 system.

All of the strains were identified as belonging to M. tuberculosis Complex by combining DNA-probe hybridisation (AccuProbe M. tuberculosis; Gen-Probe, San Diego, Calif). In vitro drug susceptibility tests against the first line drugs (Isoniazid, Streptomycin, Rifampicin, Ethambutol and Pyrazinamide) were performed by BACTEC MGT 960 SIRE and PZA (Becton Dickinson).

Results: A total of 77.56% strains were susceptible to the five first line essential drugs. Drug resistance rates of 7.28% was detected against isoniazid, 10.20% against Streptomycin, 2.33% against Rifampicin, 0.96% against Ethambutol and 2.88% to Pyrazinamide. Multidrug resistance (MDR: Resistance to both isoniazid and Rifampicin) was seen in 8 (2.43%) of the isolates. 3 (0.87%) isolates were found to be resistant to all drugs tested. 3 out of 8 MDR M. tuberculosis isolates were from patients who had immigrated to Spain and only one patient was infected by the human immunodeficieny virus. The resistance to both Isoniazid and Rifampicin decreased from 3.77% in the period of 2003–2004 to 2.35% 2007–2008.

Conclusion: Ethambutol and Streptomycin were the most and the less active drugs, respectively. According to our work, resistance to Isoniazid and Rifampicin is low in our area, specially in the last years. All reasonable efforts to prevent the spread of MDR tuberculosis must be made and maintained. It requires an efficiently working anti-tuberculosis programme to prevent resistance.

Mycobacterium genavense infections: a French national multi-centre retrospective study from 1996 to 2007


Objectives: To describe Mycobacterium genavense infections in the era of highly active antiretroviral therapy (HAART) and to provide a molecular overview of this mycobacteria using a multigenic approach.

Methods: A retrospective national survey was conducted in France. Patients with M. genavense infection diagnosed from 1996 to 2007 in a national reference laboratory were identified and their clinical, biological and microbiological data were collected with a standardised
Extensively drug-resistant tuberculosis in patients recently immigrated from Eastern Europe. Microbiological, therapeutic, and public health features

R. Manfredi*, L. Calza (Bologna, Italy)

Introduction: MDR-XDR TB is a worldwide emergency. The increased number of patients (p) immigrating from countries where health care systems could not ensure adequate drug delivery and monitoring is a major concern in Europe.

Methods and Results: During the 2nd half of y 2006 and the 1st half of y 2007 a 30-y-old male from Moldova and a 24-y-old female from Ukraine underwent very prolonged hospitalisations due to XDR TB. The 1st p, with TB known since 6y developed MDR-XDR due to frequent treatment discontinuations. On the ground of in vitro sensitivity assays, cycloserine, para-aminosalycilic acid, capreomycin, ethionamide, and linezolid were added, obtaining clinical-microbiological cure after 9 mo of hospitalisation. Three mo after discharge, our p maintained an effective 6-drug regimen on Day-Hospital basis, but 3 mo later another 5-mo hospitalisation was needed after retrieval of a positive sputum. An outpatient treatment was conducted on Day-Hospital basis for 3 mo, but positive sputum prompted a third admission lasting since 3 mo. Our 2nd p who came to Italy with a MDR TB, had an unfavourable course, and was tested in vitro for 2nd choice drugs, which suggested a cycloserine, para-aminosalycilic acid, capreomycin, ethionamide, and moxifloxacin adjunct, and achieved clinical-bacteriological cure and hospital discharge after 5 mo, despite a concurrent chronic hepatitis C which hampered liver tolerability. During the subsequent 3-mo Day-Hospital follow-up, a 5-drug association ensured a temporary cure, but 4 mo later another 3-mo admission was needed due to repeated positive sputum searches.

Conclusions: The management of the emerging MDR-XDR TB encompasses elevated clinical suspicion, diagnostic accuracy, availability of susceptibility assays of 2nd-3rd line anti-TB drugs, and adequate isolation and public health issues, when prolonged hospitalisations or protected discharges are needed. The frequent involvement of foreign immigrants is burdened by further social-economic, cultural, and administrative problems. The easy development of life-threatening, contagious MDR-XDR TB in health care contexts where low-cost anti-TB drugs are not always available, is in contrast with the huge danger and the exceedingly high costs of these episodes which need prolonged hospitalisation-isolation, and enormous technological and health care efforts. A systematic planning of the most adequate management-prophylactic measures aimed at containing-preventing XDR TB in the next future is needed.

Significant re-emerging of tuberculosis in Italy. Relationship with potential risk factors, and comparison between native residents and foreign immigrants

R. Manfredi*, S. Sabbatani, L. Calza (Bologna, Italy)

Introduction: Tuberculosis (T) is burdened by increasing morbidity-mortality rates, due to changes of epidemiologic scenario, and diffusion of resistant strains. The recent, profound modifications occurred among predisposing factors (increase mean patient [p] age, concurrent diseases, iatrogenic immunosuppression, alcoholism, drug addiction, migration, and HIV pandemic), played a key role in this process.

Methods: Among the 182 consecutive p hospitalised due to T since 1996, we compared the 101 p from Italy with the 81 immigrants from extra-European and/or developing countries, in relation to a number of risk factors, including HIV infection.

Results: Compared with immigrants, Italian p had a higher frequency of HIV-AIDS (33.7%; p < 0.001), and a predominant pleural-pulmonary involvement versus lymph node and/or disseminated T among HIV-infected p versus non-HIV-infected ones (p < 0.01). Moreover, Italians had a greater mean age (p < 0.001), and an increased frequency and a broader spectrum of predisposing conditions (positive history, chronic lung, heart, liver, kidney disease, diabetes mellitus, malignancies, and collagen vascular disease; p < 0.02), while foreigners had a lower frequency of more generic supporting factors (low income, economic-social problems, cigarette smoking, and alcohol-drug abuse; p < 0.03 versus natives). Our decade experience shed light on two different patterns of T. Local p are predominantly represented by elderly with frequent concurrent disorders and specific T risk factors, a more frequent HIV infection, and a predominant involvement of sites other than pulmonary ones, while immigrants are represented by otherwise healthy younger p, who develop prevalent lung localisations.

Conclusions: The clinicians awareness of T needs increased attention, in order to obtain a rapid diagnosis and treatment, and reduce transmission risks. The progressive integration of immigrants with local population may lead to increased risks of T dissemination, especially among the local, more vulnerable and older p population.

Trends of tuberculin skin test positivity rate among children 6–14 years old in Attica, Greece

M. Dandoulakis*, N. Roussos, D. Karageorgopoulos, N. Vatromanolakis, M. Falagas (Athens, GR)

Objective: The rising incidence of tuberculosis in developed countries is partly associated with immigration from high-prevalence countries. In Greece, a steep rise in foreign-born immigrants has been observed over the past 2 decades. We sought to estimate the prevalence and temporal trends of tuberculin skin test (TST) positivity rate among schoolchildren, in a sub-urban area of Attica, Greece.

Methods: We retrospectively analyzed the TST positivity rate (forearm volar surface induration >10mm) of schoolchildren, in the catchment area of the public primary health-care centre of Vori, over a 16-year period (from 1990–2005). TSTs were performed in the context of a national, government-directed TB screening program.

Results: A total of 11,105 records of TSTs performed in children, aged 6–14 years, were retrieved. These tests referred to 7,920 and 2,969 BCG unvaccinated and vaccinated children, respectively, as well as 120 children who had close contact with a TB-confirmed case, and 6 children with known active or latent TB. The TST positivity rate among BCG unvaccinated children was 2.0% over the whole study period; this figure declined in the second compared to the first half of the study period (1.4% vs. 2.4%, p < 0.001). The TST positivity rate among BCG vaccinated children was 63.6%, and appeared to gradually decline after vaccination.

Conclusion: The gradual decline in the TST positivity rate among BCG unvaccinated schoolchildren, despite the substantial rise in the number of immigrants in Greece, over our study period, may, at least in part, be attributed to relevant national screening and prevention measures.
Comparison of smear positive pulmonary tuberculosis in young adult and elderly patients

R. Qasemibarqi* (Qazvin, IR)

Background: Pulmonary tuberculosis remains a significant clinical and public health problem in the elderly population. To describe age-related differences in disease manifestations, a comparison of the clinical features and radiographic findings in cases of smear positive pulmonary tuberculosis among 324 patients was performed.

Design: Between January 1997 and December 2006 all patients with smear positive Pulmonary tuberculosis diagnosed at the Department of Medicine, Bo Ali Sina of Qazvine Hospital and five district health centres were recruited into the study. The following data were collected: presenting symptoms, radiographic appearance and sputum results for acid-fast bacilli. The patients were divided into two groups (254 young adult lower of 60 years and 70 elderly equal or higher of 60 years) and differences in presentation of the two groups were analyzed.

Results: Prior to treatment, symptoms occurring with a higher frequency in elderly patients included fever nightly sweating, dyspnea and haemoptysis (p less than 0.05). Symptoms occurring with equal frequency in both young adult and elderly patients included coughing, production of sputum and weight loss. Elderly patients had significantly higher incidences of negative reactions to the PPD test (p less than 0.05). Radiographic findings revealed that upper lung field infiltrates were still common in both groups, but the elderly had more lower lobe lung field involvement, and frequent cavitary lesions than younger patients (p less than 0.05).

Discussion: Since there were non-specific clinical features, false negative skin test and complex radiographic manifestations, tuberculosis was frequently not suspected in the differential diagnosis, especially among elderly patients with multiple medical problems. We suggest that physicians need to have a high level of suspicion and awareness of varied manifestations for tuberculosis, especially in elderly patients.

Application of the optimal 24 MIRU-VNTR loci set for Mycobacterium tuberculosis strains improves the correlation between strain typing and epidemiological data

J.T. Evans*, B. Taylor, D. Estephane, S. Gardiner, E.G. Smith, P.M. Hawkey (Birmingham, UK)

Objectives: The utility of DNA fingerprinting of M. tuberculosis strains and its ability to direct and focus cluster investigation has been greatly enhanced by the use of MIRU-VNTR typing. An enhanced, optimal set of 24 MIRU-VNTR loci that offers greater discrimination over the original 12 MIRU-VNTR loci and 5 ETR loci. The aim of this study was to evaluate the public health applicability, utility, and performance of the optimal MIRU-VNTR loci set in discriminating clusters defined by the original MIRU-VNTR loci that have varying levels of epidemiological links.

Methods: Six clusters containing 71 M. tuberculosis strains typed using the original set of MIRU-VNTR loci were selected for further analysis by the optimal MIRU-VNTR loci set. Epidemiological links within each of the 6 clusters were investigated retrospectively and these strains were selected for further analysis by the optimal MIRU-VNTR loci set as they contained a range of cases with varying levels of epidemiological links ranging from none found to definitive. MIRU-VNTR analysis was carried out by PCR using previously published oligonucleotides and fragment sizing by agarose gel electrophoresis.

Results: Using the original set of MIRU-VNTR loci the degree of concordance between molecular data and epidemiological data was 61%. Analysis using the additional 9 loci required to complete the optimal set of MIRU-VNTR loci increased the level of concordance to 93%.

Conclusion: The optimal set of M. tuberculosis MIRU-VNTR loci greatly increases the concordance between strain typing and epidemiological data. This further enhances the utility of MIRU-VNTR loci in DNA fingerprinting of M. tuberculosis strains as clusters identified by the original MIRU-VNTR loci set but with no apparent epidemiological links are differentiated into epidemiological relevant sub-groups whereas epidemiological relevant clusters are not significantly split by the optimal MIRU-VNTR loci set.

Comparison of Mycobacterium tuberculosis strains prevalent in the Indian Sub-continent and the UK

H.E. Smith, J.T. Evans*, E.G. Smith, R. Webber, D.A. Lannas, P.M. Hawkey (Birmingham, UK)

Objectives: Previous studies have reported the predominance of “ancient” strains in the Indian Sub-Continent (ISC) which may represent a focus of past evolution within M. tuberculosis. The study presented here analysed prevalent strains originating from the ISC and present in the Midlands, UK to compare predominant strains in the two regions.

Methods: From 4,345 M. tuberculosis isolates typed in the Midlands region of the UK, a collection of 100 prevalent representative MIRU-VNTR profiles associated with patients originating from the Indian Sub-Continent were selected for further analysis by gyrA-katG SNP analysis, RD1 and TdD1 deletion analysis, and spoligotyping. These strains were selected by using OriginsInfo software which analyses given and family names and assigns a global cultural, ethnic, and linguistic origin. SNP, RD1, and TdD1 analysis were carried out using previously published protocols using agarose gel electrophoresis. Spoligotyping was carried out using a previously published protocol for analysis on a Luminex system. Spoligotyping results were compared against a global database (spoldb4).

Results: When compared against spoldb4, 69/100 strains had defined known spoligotypes. Defined spoligotypes identified were the Central Asian Strain (n=40, East-African Indian (n=8), T (n=4), Haarlem (n=4), Bejing (n=3), and AFR1/SUX (n=6). Strains with undefined spoligotypes included probable CAS (n=19), Haarlem (n=3), LAM (n=3), EAI (n=2), X (n=1), and indeterminate (n=2). 12/100 strains possessed the TdD1 region, with all 100 strains containing the RD1 region, 76/100 strains were in PGG1, 18/100 were in PGG2, with 6/100 in PGG3. Extrapolating this data to the UK collection of 4,345 strains typed, there are 2,357 isolates originating from Southern Asia. 427/2,357 (18%) isolates are M24=2 or greater (ancient). A minimum of 935/2,357 (40%) strains may be members of the CAS family with 935/935 being modern (M24=2 or repeats).

Conclusion: Previous studies have indicated that M. tuberculosis strains prevalent in the Indian Sub-Continent are predominantly ancient. Our study of isolates in the UK originating from the ISC show that this population is predominantly modern due to the predominance of the CAS family and not the EAI family as has been reported in the ISC. This difference in prevalent strains may be due to specific strain importation from specific areas of the ISC and subsequent clonal expansion within the Midlands.

Novel mutations outside rifampicin resistance determining region associated with rifampicin resistance in Mycobacterium tuberculosis

K.H.G. Siu*, W.C. Yan (Hong Kong, HK)

Background: More than 90% of rifampicin resistance in Mycobacterium tuberculosis was shown to be caused by mutations inside the 81-bp rpoB gene. The association of mutations outside RRDR with rifampicin resistance in M. tuberculosis has not been fully elucidated.

Objective: To identify the novel mutations associated with rifampicin resistance in M. tuberculosis.

Methods: A collection of 50 clinical isolates of rifampicin-resistant M. tuberculosis were sequenced for the whole rpoB gene. The rpoB genes of the isolates with novel mutations were cloned into...
Results: PCR-sequencing of rpoB gene revealed that mutations inside RRDR occurred in 49/50 rifampicin-resistant isolates with the most common changes in codons S450L (45%), H445D (17%) and L452P (9%). Two novel mutations, I488V and I491F, were identified. The former one is hard to elucidate as the isolate also harboured the hotspot mutation, S450L. The isolate with I491F does not harbour any mutation in other region of rpoB. Transformation of the mutated rpoB gene into Mycobacterium smegmatis MC2155 rendered an increase in MIC from 32 μg/ml to 128 μg/ml. By homology, codon 491 in M. tuberculosis rpoB corresponds to Escherichia coli rpoB codon 572, which lies within the cluster II and is known to be a hotspot mutation site for rifampicin resistance in E. coli although it has not yet been reported for M. tuberculosis.

Conclusion: Result indicates that the codon 491 is probably another rifampicin-resistant determinant site for M. tuberculosis. Appropriate molecular tests should be able to detect this mutation in addition to RRDR for early and reliable prediction of rifampicin susceptibility in clinical M. tuberculosis samples or isolates.

**P604** Characterisation of pncA mutations in *Mycobacterium tuberculosis* clinical isolates from South Korea


**Objective:** Tuberculosis (TB) is a major public health problem in many parts of the world. 2 billion people—one third of the world’s population—are infected with the TB. One in 10 of those people will develop active TB. Pyrazinamide (PZA) is one of the most important and an effective first-line drug for the treatment of TB together with Rifampin (RIF). It is a produg that requires conversion into its active form, pyrazinoic acid, by the bacterial enzyme pyrazinamidase (PZase), which is encoded by the pncA. Therefore, the purpose of this study was to characterise the pncA mutations of *Mycobacterium tuberculosis* clinical isolates in South Korea.

**Methods:** DNA of specimens or *Mycobacterium tuberculosis* clinical isolates was obtained from 93 Korean patients who were clinically diagnosed with TB (Table 1). Mutations of the pncA in *Mycobacterium tuberculosis* clinical isolates in this study were identified by comparison with the pncA of the type strain *M. tuberculosis* H37Rv by using PCR-sequencing or PCR-cloning TA cloning-sequencing method. Amplifications were carried out with a GeneAmp PCR system 9600 thermocycler (Perkin-Elmer Corp., Foster City Calif). The pncA amplicons were subjected to a sequencing reaction by using a 373 Automatic sequencer and a BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems, Warrington, United Kingdom). Sequences obtained were analyzed with BioEdit software (version 5.0.9.1; T. A. Hall Software). Specific mutations will be categorised and cross-checked with the clinical data available.

Types of clinical specimens in this study

<table>
<thead>
<tr>
<th>Clinical specimens</th>
<th>No. (%) of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>36 (38.7%)</td>
</tr>
<tr>
<td>Cultures</td>
<td>27 (29.0%)</td>
</tr>
<tr>
<td>Tissue</td>
<td>11 (11.8%)</td>
</tr>
<tr>
<td>Pus</td>
<td>3 (3.2%)</td>
</tr>
<tr>
<td>Bronchial wash</td>
<td>3 (3.2%)</td>
</tr>
<tr>
<td>Bone</td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>Pleural</td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>Others</td>
<td>8 (8.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>93 (100%)</td>
</tr>
</tbody>
</table>

**Results:** Generally, 37.6% (35/93) had mutations in the pncA, including substitution, deletion and insertion as demonstrated in Figure 1.

**Conclusions:** Our results showed that the pncA mutation profile in South Korea. We found the pncA mutation rate 36.3% (16/44) and 38.8% (19/49) of *M. tuberculosis* clinical isolates in Jeju, which is located at south west from main land and other cities respectively. Also, this study is more significant of the pncA mutations in *Mycobacterium tuberculosis* incidence in South Korea compared to the previous published papers. Because this study is recently investigated and included Jeju clinical isolates which has never reported, while the last papers were published in 2001 year.

![Figure 1. Frequency and types of pncA mutation in South Korea.](image)

**P605** Mutations associated with resistance to second-line drugs in *Mycobacterium tuberculosis* clinical isolates from Lisbon, Portugal

J. Perdigão*, A. Ferreira, A. Malauquias, R. Macedo, L. Brum, I. Portugal (Lisbon, PT)

**Objectives:** Multidrug resistance (MDR) constitutes a serious problem to tuberculosis (TB) control program in Portugal. An even more serious threat is the one posed by the high rate of extensive drug-resistant TB (XDR-TB). Our laboratory has already shown that high rates of this
form of TB exist in Lisbon. Given the fact that MDR-TB and XDR-TB are currently associated with a limited number of genetic clusters, mainly Lisbon family clusters, the diversity of genetic polymorphisms conferring resistance to second-line drugs is also probably limited. In this study we intended to characterise the genetic polymorphisms associated with resistance to second-line injectable drugs and to assess the clinical isolates clonality.

**Methods:** We have analyzed 19 MDR-TB strains resistant to one or more second-line injectable drugs, collected from several hospital units across Lisbon Health Region during the year of 2005. All isolates were typed by Mycobacterial Interspersed Repetitive Units (MIRU-VNTR) and, screened for mutations in tlyA and rrs genes.

**Results:** Three different mutations were identified on tlyA gene and another three at rrs gene. Overall, 9 isolates had mutations in tlyA gene and 8 isolates had mutations in rrs gene; two isolates didn’t have any mutation in either gene. The most frequent mutations found were A1401G in rrs gene (6/19) and 755insGT in tlyA gene (6/19). We also verified that there was no overlapping of mutations from different genes. The genotyping analysis revealed that the isolates could be distributed through two different MIRU-VNTR genetic clusters: Lisboa3 and Q1. Cluster Q1 contained all clinical isolates bearing the A1401G mutation in rrs gene, while Lisboa 3 cluster contained all isolates that had the 755insGT mutation in tlyA gene.

**Conclusion:** We have identified several mutations that might be associated with resistance to different but related second-line drugs: kanamycin, amikacin and capreomycin. The two most prevalent mutations were associated with different genetic clusters, which suggests recent transmission and, ultimately, that XDR-TB transmission is taking place. The most prevalent mutations associated with injectable second-line drugs have therefore been defined, which opens the way for molecular detection of resistance to second-line drugs in the region.

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**P606** Characterisation of gidB gene in streptomycin-resistant *Mycobacterium tuberculosis* isolates in Lisbon health region

J. Perdigão*, A. Sabino, C. Milho, R. Macedo, L. Brum, I. Portugal (Lisbon, PT)

**Objectives:** Streptomycin (STP) was the first antibacterial drug introduced in the treatment of tuberculosis in 1944. With the development of further antibacterial drugs, streptomycin has become less used. Development of STP-resistance is usually explained by the acquisition of mutations in rpsL gene or in the rrs gene. Our laboratory regularly isolates STP-resistant strains without any mutation in the referred genes. Recently, mutations occurring in a tRNA methyltransferase (encoded by gidB gene) were shown to be involved in the acquisition and resistance to STP. In this study, we examined the gidB gene of STP-resistant isolates in search of mutations that may explain the acquisition and STP low-level resistance on these strains.

**Methods:** We have analyzed by sequencing and/or endonuclease analysis the gidB gene of 57 STP-resistant clinical isolates and 30 STP-susceptible clinical isolates, recovered in 2005 and 2006 from different hospital units. The entire rpsL ORF of all isolates was amplified and screened for mutations by endonuclease and sequencing analysis. All clinical isolates were also genotyped by MIRU-VNTR.

**Results:** The gidB gene of 19 STP-resistant isolates was sequenced and two missense mutations, A80P and F12L, were found in 5 and 1 out of 19 isolates, respectively. We have found that these gidB mutations were only present in isolates without rpsL mutations. The remaining isolates were screened by endonuclease analysis for mutations A80P and K43R in gidB and rpsL genes, respectively. Overall, mutation A80P in gidB gene was found in 10/37 STP-resistant isolates; 11/14 STP-susceptible multidrug resistant isolates; and, none of 16 pansusceptible isolates. GidB mutation A80P was also associated with MIRU-VNTR genetic cluster Q1, although an independent occurrence has been identified.

**Conclusion:** We conclude that gidB mutations may in fact explain the high number of STP-resistant strains with no mutation in rpsL or rrs, isolated in our laboratory. These mutations probably confer STP low-level resistance that may pass undetected in regular drug susceptibility testing. The independent occurrence suggests however, that the acquisition of such mutations present an adaptive advantage.

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**P607** Structural and biochemical study of new KatG mutations found in clinical strains of *Mycobacterium tuberculosis* resistant to isoniazid

F. Brossier*, M. Bouldinet, W. Sougakoff (Paris, FR)

**Objectives:** Resistance to isoniazid (INH) is mainly due to acquisition of mutations in the KatG protein (the catalase-peroxidase), particularly in position 315 (Ser315Thr). In previous studies, we evaluated the performance of a diagnostic strip, MTBDRplus, enabling rapid identification of S315T in strains of *M. tuberculosis* resistant to INH. In the course of this study, we identified new mutations in KatG, the role of which has never been characterised before. For these mutations, structural and biochemical studies have been undertaken to establish their contribution in resistance to INH.

**Methods:** KatG genes coding for the wild-type protein and six new mutants A162E, D189G, H270R, Q461P, G494D and F658V have been included. Expression and purification of KatG in *Escherichia coli* was carried out by cloning the katG genes in the pET30 expression vector. The crystal structure of the *M. tuberculosis* KatG protein (Zhao X et al., code-PDB 2CCA) was used to model the position and the consequences of the new mutations in the KatG protein.

**Results:** The KatG protein contains a covalently bound heme surrounded by a proximal and a distal pocket. Of all the mutations studied, H270R, which was identified in a clinical strain of high level of INH resistance (INH-R), appears to be of great interest since H270 is part of the proximal pocket and is covalently linked to the heme. Thus, H270R causes loss of the covalent bond linking the heme and, consequently, of the catalytic activity of KatG. The mutation A162E, linked to a high level of INH-R, is located in a alpha-helix situated close to the distal pocket and creates steric hindrance in this region. Two others mutations (F658, Q461P), found in strains showing a low level of INH-R, are located in alpha-helices positioned far from the heme. F658V leads to steric hindrance, while Q461P likely contributes to the destabilisation of the alpha-helix secondary structure. Finally, D189G and G494D, conferring low and high-level of INH-R, respectively, modify the pattern of ionic interactions in regions located far from the heme pocket, and their role in the KatG protein is less obvious.

**Conclusions:** These results shed light on the importance of KatG mutations other than S315T in resistance to INH and will be integrated in a global strategy involving molecular modeling and biochemical studies to increase our ability to predict resistance to INH, a goal particularly important to improve the treatment of TB patients.
Results: Only the mutation A63P previously identified for M. tuberculosis was found in the mutants selected from H37Rv whereas we identified the mutations D32G, L59V, E61D, A63P and I66M in the mutants selected from clinical strains. Thanks to the model of the structure of the c ring of M. tuberculosis we can see that the 4 amino acids implicated in the resistance to TMC207 are all in the close vicinity of E61 implicated in the transfer of proton and also in the resistance, and they form a putative binding pocket where the bulky brome atom of the drug is deeply accommodate. We also highlighted another major interaction between the drug and the c ring which is the presence of H-bonds between residues D32, E61 and TMC207. Accordingly, complementation studies in M. smegmatis revealed that amino acids substitutions D32G/V and E61D efficiently increase the level of resistance to TMC207.

Conclusion: Our objectives are to define, in as much detail as possible, the binding site of TMC207. To achieve this goal, we have developed an efficient tool combining complementation assays and molecular modelling. Our initial results show that amino acids D32, L59, E61, A63 and I66 make a deep binding pocket where the brome atom can accommodate and the binding of TMC207 is also stabilised by H-bonds with residues D32 and E61.

Molecular epidemiology and antimicrobial susceptibility of Mycobacterium abscessus isolated from patients with cystic fibrosis

C.A. Burnham*, W.M. Dunne (St. Louis, US)

Objective: Mycobacterium abscessus is a rapidly growing mycobacterium that is ubiquitous in the environment. While M. abscessus is commonly associated with skin and soft tissue infections, it is also capable of colonising and causing disease in the respiratory tract. One group with a propensity for colonisation with M. abscessus is patients with cystic fibrosis (CF). The objective of this study was to determine if patients infected with M. abscessus harbour the same strain over time, as well as to determine if patients cared for at the same centre share strains. The antimicrobial susceptibility profile of the isolates was also obtained to determine if chronic colonisation was related to resistance to typical antimicrobial regimens for this organism.

Methods: Mycobacterium abscessus from CF patients who had the organism isolated from at least 3 separate respiratory cultures collected from 2004 to 2007 were included, corresponding to 35 isolates from 9 patients. The organisms were cultured according to standard protocols in the Barnes-Jewish Hospital Mycobacteriology laboratory. DNA was extracted and strains were compared using repetitive sequence PCR (REP-PCR). DiversiLab Bacterial Barcodes software was used to compare banding patterns and determine the similarity index (SI) between the isolates. Antimicrobial susceptibility testing was performed using a Trek Diagnostics Sensititre broth microdilution panel for rapidly growing mycobacteria in accordance with NCCLS document M24-A.

Table 1. Summary of antimicrobial susceptibility testing results

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Antimicrobial agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible (n)</td>
<td>CLA L2D TOB FOX AMI SMX DOX CIP</td>
</tr>
<tr>
<td>Intermediate (n)</td>
<td>35 21 5 6 26 0 4 1</td>
</tr>
<tr>
<td>Resistant (n)</td>
<td>0 11 14 19 3 0 5 1</td>
</tr>
</tbody>
</table>

Results: The REP-PCR analysis of sequential strains isolated from individual patients showed a high degree of similarity, suggesting that once the organism is acquired, a patient remains colonised by a single clone, rather than clearing the organism and subsequently becoming infected with a different strain. The M. abscessus isolated from 3 of the patients had unique REP-PCR patterns, but there were 3 separate sets of 2 patients with shared patterns (SI > 95%). The results of the antimicrobial susceptibility testing are in Table 1. Of note, all of the 35 isolates tested were susceptible to clarithromycin.

Conclusions: The results of this study suggest that CF patients from which M. abscessus is repeatedly isolated are chronically colonised with a single clone. In vitro susceptibility may not correlate with clinical response as all isolates tested in our study are susceptible to clarithromycin, the antimicrobial agent of choice, yet the patients in our study do not eradicate the organism.
Incidence, clinical and epidemiological risk factors, and outcome of drug-induced hepatitis due to anti-tuberculous agents in new TB cases

P. Baghaei Shiva * (Tehran, IR)

Background: Drug-induced hepatitis (DIH) is an important issue in TB treatment. We intend to assess the incidence, risk factors and outcome of hepatitis due to anti-TB drugs.

Methods: The study is carried out at the national TB referral centre 2006–2008 including all documented new case TB patients. All patients received standard anti-TB treatment. If DIH occurred, all drugs were discontinued; and re-initiated after LFT normalisation in a stepwise way.

Results: Of total 761 patients 99 (13.0%) patients developed drug-induced hepatitis during anti-Tb treatment. There was no difference in sex, nationality, smoking or opium use history between in Hepatitis group and control group (P value >0.05).

DIH was significantly higher in patients aged above 65 years. (p = 0.019).

The mean duration of DIH from the beginning of treatment was 17.53±19.42 days (Median = 12; 1–125 days). Also, the mean of the time elapsed from DIH till the LFT normalisation was 19.42 days (Median = 12; 1–125 days). Also, the mean of the time of DIH was 10.26±17.53 days (Median = 9; 0–32 days).

Anorexia, nausea, vomiting, abdominal pain, jaundice, diarrhoea, decrease level of consciousness, and fever were significantly higher in DIH patients. 13 (13.4%) of the patients in DIH group died while death occurred just in 21 (3.2%) of cases in the control group. (p < 0.001, 95% CI = 2.26–9.70, odds ratio = 4.7).

Conclusion: Our study indicated that DIH most often occurs during the first two weeks of anti-TB treatment. DIH development is associated with old age, certain clinical manifestations, and higher death rates.
Clinical epidemiology of nosocomial infections

**Do splenectomized patients for trauma suffer from more early postoperative infections?**

I. Pehuela*, J. Palomino, M.D. Rincón, J.M. Dominguez, F. Marillo, J. Puchón (Seville, ES)

**Objectives:** Little is known about the effect of splenectomy for trauma on early postoperative infections. The purpose of this study was to determine if splenectomy increases the early mortality and postoperative infections in trauma patients.

**Methods:** We review all trauma patients undergoing splenectomy from January 20006 through December 2007. Each splenectomy patient (SP) was matched with two trauma patients without splenectomy (WSP) based on age, gender, injury severity score (ISS), and hospitalisation date, by using a design of matched cohort study. The primary outcome was the appearance of an infectious complication (pneumonia, bacteraemia, and urinary tract infection [ITU]), and the secondary outcome was in-hospital mortality, both during the first 30 days. Statistical analysis was performed using SPSS 16 version. The log-rank test was used to compare the mortality, and timing of apparition of infections.

**Results:** There were 20 SP and they were matched to 40 WSP. SP and WSP were similar regarding age (33.6±11.2 vs 33.6±10.7; p=0.95), male gender (100% vs 100%) and ISS (26 [29.2–19.2] vs 25.5 [38–19.6]; p=0.84). Pneumococcal 23-v (mean=4.44 days after) and Hib (mean=0.50 days after) vaccines was administrated to 85% and 65% SP, respectively. SP developed pneumonia (15% vs 27.5%; p=0.34; RR=0.58 [0.19–1.69]), bacteraemia (10% vs 7.5%; p=1.00; RR=1.22 [0.39–3.81]) and ITU (0% vs 12.5%; p=0.15). Isolated microorganisms in SP with pneumonia were Gram-negative bacilli 2, unknown 1, and in WSP 3, Acinetobacter spp 2, others 4, and unknown 2. In SP with bacteraemia P. mirabilis 1 and S. saprophyticus 1, and in WSP S. epidermidis 2 and S. aureus 1. There were no ITU in SP. In WSP with ITU, Gram-negative agents 4. The mortality rate in SP was 10% vs 5% in WSP (p=0.59; RR=2.11 [0.27–16.21]). Statistical differences were no found in survival curves as for time to appearance of death (log-rank 1.38; p=0.24), pneumonia (log-rank 1.16; p=0.28), and bacteraemia (log-rank 0.24; p=0.62) in SP vs WSP during the first 30 days.

**Conclusion:** In patients vaccinated with pneumococcal and Hib vaccines and an ISS mean score of 26, SP for trauma is not associated with an significant increase of postoperative infections (pneumonia, bacteraemia) neither higher mortality than WSP.

**Early intravenous to oral antibiotic switch therapy is effective in the treatment of infected total hip replacement**

E. Darley*, G. Bannister, A. Blom, A.P. MacGowan, S. Jacobson, W. Alfonsan (Bristol, UK)

**Objective:** To determine outcomes for an antibiotic regimen using early switch to oral antibiotic (AB) for treatment of infected total hip replacements (THR) treated by either 1-stage or 2-stage procedures.

**Methods:** Cases of infected THR were identified from the microbiology records held on all orthopaedic infections in a 24 month period in North Bristol NHS Trust. Diagnosis was made by microbiological culture of theatre specimens and findings at operation. The total number of THR operations was also determined. Data on organisms cultured and AB treatment regimens were recorded from orthopaedic cases notes, microbiology patient and pathology computer records. AB treatment regimens were tailored for each patient by a medical microbiologist, according to culture and sensitivity results and history of previous THR infection. A standard approach of 10–14 days intravenous (iv) AB followed by a switch to oral ABs either for 6–8 weeks until 2nd stage re-implantation or for 6–26 weeks following a 1 stage procedure, was used. The exact date of oral switch and ultimate AB duration was determined by clinical resolution and the CRP Outcome was recorded as no microbiological or clinical evidence of relapse of infection, relapse after completing AB course, or unknown.

**Results:** In 24 months 1854 THR elective operations were performed, 1% for infected THR. 19 patients underwent 2-stage THR, 17/19 were treated with 14 days iv ABs followed by oral ABs for 4–8 weeks. 2/19 patients were treated with iv ABs for the whole duration, 1 for a resistant pseudomonas and 1 patient with co-morbidities who remained in hospital. None of the 19 patients have relapsed. 6 patients underwent 1-stage THR, 4 had 2 weeks iv then 6–26 weeks oral ABs, with no relapse. Case notes are unavailable for the other 2 patients, neither has represented to this hospital Trust for further treatment. Follow up duration for all cases, to date, is 12–24 months.

**Conclusions:** 17/17 patients treated for infected THR with 2-stage replacement and an AB regimen incorporating early switch from iv to oral ABs have had to date a successful outcome. Early oral AB switch therapy is effective and has an important role enabling patients to return to independence after revision surgery and avoid complications of prolonged iv access.

**Do cardiac centres in the United Kingdom have a standard policy for antibiotic prophylaxis in cardiac surgery? First clinical audit in last 10 years**

A. Guleri, S. Hosmanc*, J. Zacharias (Blackpool, UK)

**Objectives:** Antibiotic prophylaxis (AP) is known to reduce surgical site infections [SSI] post cardiac surgery. There appears to be variation in the choice of antibiotic/s, dose and duration between surgeons and across centres. Preliminary to drafting AP guidelines for surgery, Microbiology and cardiac surgery at Lancaster carhde centre, Blackpool carried out this audit of practice across all cardiac centres in England.

**Method:** An online questionnaire was sent to consultant surgeons in all the 36 cardiac surgery units in England. 26 responses obtained representing all geographical regions of the country.

**Results:** All centres use prophylactic antibiotic/s. 50% [13/26] use a combination of two antibiotics while the rest use a single agent. 92.3% [12/13] use cefuroxime as a single agent and 7.8% [1/13] use flucloxacillin. Different combinations of seven antimicrobial agents used in 50% centres include cefuroxime [73.1%], flucloxacillin [23.1%], gentamicin [19.2%], vancomycin [15.4%], and Teicoplanin [11.5%]. The antibiotic/s are administered at induction of general anaesthesia (73.1%), just before the skin incision (19.2%) or along with the premedication in the ward (7.7%). Duration of use – Over 24 hrs (76.9%); more than 24 hrs (19.3%) and as a single dose (3.8%). The trust policy addressing prophylactic antibiotic in cardiac surgery is followed by 88.5% [23/26]. The policy formulation has contribution from cardio surgeons (95.7%), microbiologists (87%), infectious disease consultant (26.1%), cardiac anaesthetists (21.7%) and pharmacists (4.3%). The estimated sternal wound infection rates were <1% in 7 (26.9%), 1–3% in 18 (69.2%) and >3% in only 1 (3.8%) centres. As per 24/26 responses on MRSA infections per month, it is <1% in 21 (87.5%) and >1% in 5 (12.5%). The clodziostrol difficile infection rates per month (23/26 responses) are <1% in 12 (46.0%) and >1% in 9 (39.1%).

**Conclusions:** There is evidence of reduction in rates of SSIs with use of antibiotic prophylaxis in cardiac surgery. However, there is paucity
of randomised controlled studies comparing choice of agents, single or combination of agents, duration of use (single dose, <24 h or <48 h) and rates of infections. This clinical audit has revealed variation in practice across cardiac centres in England and lack of correlation to SSIs. The results of this national audit of practice, recent NICE guidance on SSIs and local HCAI data will help frame revised trust guidance on antibiotic prophylaxis in surgery.

Figure: Prophylactic antibiotic usage in cardiac surgery.

**P617** Rapamycin, used in the prevention of in-stent restenosis, has antichlamydia-activity

Y. Yan, S. Silvennoinen-Kassinen, M. Leinonen, P. Saikku (Oulu, FI)

Objective: Rapamycin coated eluting-stents have been used recently in humans to reduce the risk of in-stent restenosis [1]. Serological study suggested that *C. pneumoniae* could play a role in the pathogenesis of restenosis [2]. Here we examined the effect of rapamycin on the growth of *C. pneumoniae* in vitro.

Methods: *C. pneumoniae* CWL029 or *C. trachomatis* L2 were inoculated onto the HL cell monolayers by centrifuge. Infected HL cells were incubated for 48 h (*C. trachomatis*) or 72 h (*C. pneumoniae*) at 35°C and 5% CO2. During the incubation, 23, 11 or 7 ug/ml rapamycin was present in the culture medium continuously or for 8-hour periods: 0–8, 8–16, 16–24, or 24–32 h. *C. pneumoniae* infected cells cultured with 0, 11, 7 or 3.5 ug/ml rapamycin respectively were repassaged to fresh HL cell monolayers with centrifuge and incubated for another 72 h. The infected cells from both passages were checked by a fluorescent microscope or an electron microscope.

Results: The growth of both *C. trachomatis* and *C. pneumoniae* was inhibited by 74–94% when 23 ug/ml rapamycin was present during 0–8 h and 8–16 h after inoculation, but effect on *C. pneumoniae* lost after 24 hours although still effective on *C. trachomatis* (p < 0.01). Other concentrations were not effective. Continuous presence of 11 and 7 ug/ml rapamycin inhibited the growth of *C. pneumoniae* by 80% (p < 0.01) and 27% (p < 0.05) respectively, but 3.5 ug/ml rapamycin had no significant effect. The diameter of the inclusions decreased from 12.6±1.75 um in controls (a.) to 3.4±1.3 um in those under influence of 23 ug/ml rapamycin during 8–16 h, or continuously presented 11 ug/ml rapamycin (b.) in which there are fewer chlamydial particles and fewer matured EBs (b.). 11 ug/ml rapamycin presented in first passage caused the reduction of the growth of *C. pneumoniae* to 57% at first passage and to 24% at second passage (p < 0.05).

Conclusion: It has been shown that roxithromycin prevents restenosis in patients with high *C. pneumoniae* antibody titres [3] suggesting that antibiotics effective against chlamydia might have beneficial effects. We showed here that rapamycin exhibits antichlamydial activity on *C. pneumoniae* in HL cell cultures. Thus, the beneficial effects of rapamycin in the prevention of in-stent restenosis might partly be explained by its antichlamydial properties.

Reference(s)


**P618** Characterisation of LD- and EVD-associated meningitis/ventriculitis in neurosurgical patients

S. Scheithauer*, U. Bürgel, H. Schulze-Steinen, H. Haefner, S. Lemmen (Aachen, DE)

Objective: Data on drain-related infection rates [IR] are rare. To determine IR and to characterise patients with drain associated infections [DAI] and identify possible risk factors, we conducted a prospective surveillance study at the neurosurgical ICU of the University Hospital Aachen.

Methods: All patients admitted between January and December 2007 were enrolled. DAI including symptoms and laboratory results, kind and duration of drainage utilisation [DU], acute and underlying diseases, therapy and outcome were recorded.

Results: During 4086 patient-days (375 patients) and 2290 drainage-days [DD] (496 LD-days, 1794 EVD-days) in 149 patients 28 cases of meningitis (12 with LD, 15 with EVD, one with both) occurred. Thus resulting in an overall IR of 12.23/1000 DD, 26.21/1000 LD-days and 8.92/1000 EVD-days, respectively. In 19/28 (68%) infections a pathogen could be detected, in 16/28 (57%) cases the clinical criteria for a drainage associated infections were fulfilled. Coagulase-negative staphylococci were the main pathogen (8/19 culture positive cases; 42%). Decreased glucose level (CSF) was the most common laboratory parameter (21/28; 75%).

Figure 1. Incidence of LD- and EVD-associated meningitis.

Meningitis was more often in patients with SAB °V (6/28; 21%; OR: 6.33), ICB following trauma (3/28; 11%; OR: 3.5), in those
with diabetes mellitus (5/28; 18%; OR: 3.1) or neoplasms (10/28; 36%; OR: infinity) and in patients with LD versus EVD (OR: 2.06). Association with SABV (p < 0.005) and neoplasms (p < 0.000) were highly significant.

The average of DU was 8.6 (median: 6) days for LD and 15.0 (median: 14) days for EVD, respectively. For correlation of incidence of meningitis and duration of LD and EVD see figure 1.

Conclusions: Benchmarking/Comparison of these results with earlier studies is difficult since different surveillance parameters and case definitions were used in previous investigations. This study represents provides data on drain-associated meningitis rates in combination with identifying associations and/or possible risk factors. Moreover the reliability of various clinical and laboratory parameters commonly used was evaluated.

**Ventricular assist device-related infections**

A. Gkouziouta*, P. Sfrakis, L. Louca, S. Adamopoulos, V. Voudris, G. Saroglou, P. Alcizatos (Athens, GR)

**Objectives:** Heart failure remains a leading cause of death in developed countries despite medical management. Heart transplantation (HTx), a proven lifesaving intervention, is limited by donor availability. Ventricular assist devices (VADs) provide temporary support for patients with severe heart failure until transplantation or more seldom myocardial recovery. Assist devices may be used permanently for those ineligible for transplantation having demonstrated a survival benefit and an improved quality of life. Infection, in 18%–59% of cases, may involve any component of the device causing substantial mortality and morbidity.

**Methods:** We retrospectively reviewed the medical records of 39 patients with implantable VAD at the Onassis Cardiac Surgery Centre supported longer than 72 hours, from February 2003 through September 2008. Infection types included primary bacteraemia, septicaemia, endocarditis, pocket, driveline and exit site infection.

**Results:** In 39 patients supported with VAD, 9 developed 14 VAD related infections. Primary bacteraemia in 4 patients, VAD endocarditis in 3, while driveline and exit site infection occurred in 7 patients. Duration of VAD support was longer in infected patients (2648 days) vs. uninfected ones (1500 days). Pathogens identified: Staphylococcus epidermidis (4), Pseudomonas aeruginosa (4), Klebsiella pneumoniae (2), Acinetobacter baumannii (2), Proteus mirabilis (1) and Candida parapsilosis (1) were also identified. Five patients were successfully treated with i.v. antibiotic usage. Four patients were urgently transplanted due to septicemia from multi antibiotic resistant nosocomial pathogens not responding to antibiotic treatment. One patient died from polymicrobial bacteraemia with Klebsiella pneumoniae, Stenotrophomonas maltophilia and Candida albicans. We were unable to transplant him due to lack of donor heart.

**Conclusions:** Infection remains the most common complication for VAD application. Development of appropriate strategies is essential such as continuous clinical surveillance, continuous patient clinical surveillance and infection control preventive measures are essential in a HF-VAD unit to prevent and manage device related infections in the MDR patient era.

**Risk factors for colonisation by colistin-resistant Klebsiella pneumoniae in critically ill patients**

F. Kontopidou, D. Plachouras, G. Koukos, E. Papadomichelakis, I. Galani*, A. Antoniadou, G. Poulakou, A. Armaganidis, H. Giamarellos (Athens, GR)

**Introduction:** Emergence of colistin resistant Gram-negative bacteria, and especially strains of Klebsiella pneumoniae, in the ICU severely limits our treatment choices in critically ill patients. The aim of this study was to investigate the risk factors for colonisation by such strains.

**Methods:** The study was performed in a 12-bed University General ICU from November 2003 to December 2006. Empirical antimicrobial treatment was guided by weekly active surveillance of patients’ floras. All specimens were cultured in MacConkey agar plates containing antibiotics in order to focus on resistant pathogen detection. Colistin resistance was defined by Etez according to BSAC breakpoints (<4 mg/L). Demographic and clinical data of the patients were recorded.

Risk factors for colonisation by CRKP were assessed by univariate and logistic regression analysis.

**Results:** 150 patients (mean age 65.1 years) with mean APACHEIII score (SD) 18.7 (7.8) and mean days of hospitalisation (SD) 76.0 (54.0) were included in the study. 29 (19.3%) patients were colonised with CRKP. Among these patients seven (24%) developed an infection by CRKP with fatal outcome in six of them. The median number of hospitalisation days was 64.5 in the group colonised with colistin resistant isolates compared to 34 days in the non-colonised group (p<0.01). Mean APACHEIII score was 19.6 and 18.4 respectively (NS) in the two groups. Administration of colistin was significantly associated with colonisation by CRKP strains (p<0.001). The median duration of colistin treatment was 23.0 and 14.7 days among patients colonised and not colonised with CRKP respectively (p NS). Among the patients with CRKP 26 (89.7%) had previously received colistin. 15 (16.1%) of patients who had received piperacillin/tazobactam were colonised by CRKP compared to 14 (24.6%) of patients who had not received this combination (p NS). In the multivariate analysis model the only significant risk factors for colonisation with colistin and not colonised with CRKP were administration of colistin (p<0.005, OR 6.7) and piperacillin/tazobactam (p<0.017, OR 0.32).

**Conclusions:** Colistin use is the only significant risk factor associated with the emergence of resistant Klebsiella pneumoniae strains, jeopardising treatment choices in the ICU. Unnecessary or prolonged administration of colistin should be avoided.
wards. In adult IC units, pneumonia and septicemia represented resp. 51% and 20% of NI.

The annual number of patients suffering from a NI in Belgium was derived from the results of the PPS, and estimated to be between 103,000 and 116,000 or 0.97 to 1.10% of the Belgian population.

Conclusion: For the first time in 25 years, a national study determined the burden of NI to patients and society as comparable to neighbouring countries. The use of an IT expert system facilitated participation and ensured higher standardisation.

P622 National investigation into the infection control practices in surgery in Belgium
B. Goral*, A. Lenez, B. Heyneyn, M. Costeron behalf of the Belgian Federal Platform for Hospital Hygiene and BAPCOC

Objectives: Belgian regulations compel acute care hospitals to have local regulations on infection control (IC) in operating suites (OS). However, since no national minimal IC standards exist, the federal platform for hospital hygiene (FPHH) suspected a different approach in Belgian hospitals. In order to evaluate the present IC practices in our country, the FPHH performed a national survey evaluating the extent to which internationally suggested IC precautions are actually defined, carried out and monitored in Belgian OS.

Methods: A working party of the FPHH established an inventory of essential IC precautions prescribed in national guidelines in France, the Netherlands and the United States and grouped the series into different categories: architecture/structure, cleaning, per-operative procedures, sterilisation, logistic activities and surveillance evaluating postoperative wound infections (POWI). A questionnaire was sent to all Belgian acute care hospitals, examining the local implementation of – and compliance with – each IC measure.

Results: In this national survey, 92 acute care hospitals (81%) provided data on 168 OS. Compliance with basic IC measures like closing doors during surgery, awaiting wound closure before clearing the OR, wearing gloves and masks, antimicrobial prophylaxis and even standard procedures were sometimes included. Finally, large differences existed regarding which questionnaires and interviews are designed to collect appropriate data.

Results: 65 NRIC users signed up for the impact evaluation. Of these 32 completed pre and post visit questionnaires of which 72 sets were matched for analysis. On arriving at the NRIC library participants were asked to login with a unique username and password for the purposes of tracking their activity during the study period. NRIC had an impact on user knowledge in 52.8% of visits. Most visits were to seek evidence to either support existing knowledge or practice (n=28) or extend existing knowledge (n=9). Other reasons included seeking general information about a subject (n=10), looking for new resources or news (n=14), looking for training information or educational materials (n=4) or searching for specific documents (n=4). Reasons for no impact were that not enough information was found or the user couldn’t access the document. NRIC has a positive impact in many areas of user work including policy development, training and education, implementing changes in practice and business case or proposal preparation (See Fig).

Conclusion: This study has shown that NRIC is a popular, easy to use, library that is having a positive impact on user knowledge and work. However, further sustained investment is required if NRIC is to fulfil its potential as a one-stop resource in the infection prevention and control community. Raising awareness is key to encouraging the involvement of this community in developing NRIC and ensuring that any development is in-line with user needs.

Surveillance of nosocomial infections

P624 Review of methods of national prevalence surveys of healthcare-associated infections in 17 European countries
C. Suetens*, A. Ammon, K. Weist, L. Sodano, D.L. Monnet (Stockholm, SE)

Objectives: In order to prepare a common protocol for a EU-wide point prevalence survey (PPS) of healthcare-associated infections (NI) in 2010–2011, a methodological analysis of current national protocols in 17 European countries was carried out.

Methods: Methodological data were extracted from papers, full reports or protocols published in international or national scientific journals, on the internet or from copies obtained from national PPS coordinating centres when not available elsewhere. Keywords used for MEDLINE and Google searches were: prevalence combined with nosocomial, hospital, hospital-acquired and healthcare-associated infections.

Results: The percentage prevalence of HCAI varied from 3.5% to 9.9%. The main differences are summarised in table 1. The first difference concerns the use of different case definitions. Secondly, all criteria of the case definition had to be present on the day of the survey in some studies while in others criteria were looked at for the entire infection episode. The strict application of the criteria was either controlled, e.g. during analysis, or left to the investigators’ judgment. Third, included infection types were sometimes limited to the major types only (urinary tract, bloodstream, lower respiratory tract, surgical site infections). Fourth, specific infection types included different subtypes (e.g. exclusion of asymptomatic bacteriuria). Fifth, infections acquired in other hospitals were sometimes included. Finally, large differences existed regarding...
A national point prevalence measurement of healthcare-associated infections in somatic care in Sweden, 2008


Objectives: The objective was to perform a nation wide web-based point prevalence measurement (PPM) of healthcare associated infections (HAI) and risk factors. The PPM is part of the national patient safety initiative and will be performed twice during 2009.

Method: A national cross sectional PPM of HAI was performed within a two-week period in November 2008. Demographic data, five defined risk factors for HAI and antibiotic therapy for all admitted patients was recorded by the staff of each ward. HAI was recorded in relation to 18 pre-defined diagnosis groups. Type of HAI was referred to as postoperative, device or drug related, and others.

Results: 101 hospitals included 22,746 patients, close to all admitted patients in Sweden. 20,131 were admitted in somatic wards. 1,203 were children and 54% women. 52.1% were admitted to specialities treated for HAI, corresponding to 89% of all HAI.

Conclusions: The PPM method was successfully introduced including almost all patients in Swedish hospitals. The results give us specific knowledge of HAI including the role of the risk factors. The facilitation of the national patient safety initiative is important as well as the tutorial aspects of self-assessment of HAI. Local results are available for immediate analysis.

Epidemiology and outcome of C. difficile infection in a large UK teaching hospital

H. White, D. Bell, J. Reid, C. Walker, M. Wiselka* (Leicester, UK)

Objectives: C. difficile infection is an important healthcare associated infection. University Hospitals of Leicester (UHL) experienced a significant increase in cases over the three years until 2006, coinciding with the introduction of the ribotype 027 strain. A C. difficile cohort isolation ward was opened in April 2007. The aims of this study were to review the epidemiology and outcome of infected patients and the impact of the Isolation Unit.

Methods: Clinical information on cases of C. difficile infection was collected prospectively using a standard clinical proforma. A total of 492 outcome forms were completed between October 2006 and March 2008. Patients were managed according to an agreed protocol during this period.

Results: The median age of patients with C. difficile infection was 77 years. 70% were admitted from their own homes. In 81.6% of cases this was their first admission with C. difficile, but 18.6% of patients were admitted with recurrent disease. In hospital mortality was 58% and mean length of stay was 40 days. 73% of those discharged returned to their own home. 30% of patients were on a proton-pump inhibitor at the time of diagnosis of C. difficile and 87% had received antibiotics within the last 2 months.

Initial treatment with 10 days of oral metronidazole led to resolution of diarrhoea in only 34% of cases. Subsequent treatment with oral vancomycin (125 mg four times daily) led to resolution in a further 51%. Approaches used to treat those patients who did not resolve after metronidazole/vancomycin included pulsed therapy and intracolonic vancomycin.

The opening of the C. difficile Unit resulted in an 80% reduction of new cases of C. difficile infection identified in the Hospital Trust. Patients admitted to the Unit had significantly lower mortality and length of stay. Conclusions: This is one of the largest prospective studies of the outcome of C. difficile infection and highlights the impact of infection and associated in-hospital mortality. The outcome of metronidazole treatment was disappointing with vancomycin appearing to lead to resolution in a greater proportion of cases. There is currently no consensus on treatment of C. difficile infection which is recalcitrant to metronidazole and vancomycin therapy. The opening of the cohort ward and other measures implemented by the Trust resulted in a dramatic and sustained fall in the incidence of C. difficile with a significant improvement in clinical outcome.

English voluntary surveillance scheme for Clostridium difficile infections: seasonal variation by age group (January 2000 – December 2008)


Objectives: This paper describes apparent age variation in seasonality of C. difficile infection (CDI) in hospital and community associated cases in England.

Methods: Data on CDI reported by National Health Service (NHS) laboratories in England are collected by both voluntary and mandatory reporting schemes. This paper analyses reports sent to the voluntary surveillance scheme for the period January 2000 to December 2008. The reports are from specimens taken from patients in healthcare facilities and the community. The dataset includes patient sex, sounex, age and specimen date.
A case-control study during an outbreak of Surveillance of nosocomial pneumonia and bloodstream Pneumonia antifungal agents (OR 8.1). Individual comparison of CDI due to types of second generation cephalosporins (OR 12), clindamycin (OR 11) and were nasogastric intubation (OR 2.7), recent admission (OR 2.1), use and 162 patients without diarrhoea. Independent risk factors for CDI with other types. Controls consisted of 77 non-CDI diarrhoeal patients of whom had CDI with type 017, 46 (27%) with type 027, and 65 (39%).

Results: There was a four fold rise in reported CDI in the 75 years and over age group between 2004 and 2007, followed by a reduction in 2008. Marked seasonality with peaks in the January to March (Quarter1) from 2004 to 2007, is predominately seen within the oldest age and 65–74 groups (30% of annual cases occurring in this quarter). Regular seasonal patterns are not discernable in the younger age groups (2–14, 15–44 and 45–64 years). CDI in 2008 shows rapidly decreasing counts and lacks a peak in the first quarter of the year, which may be due to a mild winter. There is marked variation between regions: Q1 peaks have been sustained high since 2004 for the ≥75 years age group in the South Western, North Eastern, East of England and Yorkshire and Humberside Regions of England. These differences may be due to one or more of a number of reasons: variable ascertainment, epidemiology effect of different strain types and the consequent variation of associated risk factors.

Conclusions: There is no current explanation of the seasonality seen in the first six months of the year. Further investigation of the association of antibiotic usage linkage and strain typing data is required to test the hypothesis that pneumonia diagnosis and treatment with broad spectrum antibiotics could explain this seasonality.

**S628**

**A case-control study during an outbreak of Clostridium difficile infections due to PCR ribotypes 027 and 017 occurring simultaneously in one hospital in the Netherlands**


Objectives: Outbreaks of Clostridium difficile infection (CDI) due to PCR ribotype 027 (type 027) are emerging worldwide, whereas some European countries report an increase of toxin A negative PCR ribotype 017 (type 017). We encountered a unique outbreak due to types 027 and 017 occurring simultaneously in a 1100 bed teaching hospital in The Netherlands.

Methods: From May 2005 through January 2007, an outbreak of CDI occurred with a peak incidence of 85 per 10,000 admissions. A case control study was performed with two different control groups. Risk factors and outcome parameters were compared in multivariable analysis, using age, sex, medical specialty, co-morbidity score and concomitant use of drugs and antibiotics as co-variates. All isolates from CDI patients were investigated by PCR-ribotyping and multi locus variable number of macrolides (OR 9.4), clindamycin (OR 2.3) and immunosuppressive agents (OR 5.0) as risk factors for type 017. Patients with type 027 used significantly less clindamycin (OR 0.3). The overall mortality at 1 year follow-up was significantly higher among CDI patients, compared to both patients with non-CDI diarrheal and non-diarrheal patients (38%, 29% and 20%, respectively). The overall mortality among patients with types 017 and 027 was higher when compared to patients with other types (46%, 49% and 23%, respectively). MLVA showed clonal spread of types 017 and 027 throughout the hospital, where clones could persist on wards for more than a year, despite thorough disinfection.

Conclusions: In this unique outbreak in one hospital, distinct risk factors were found for types 017 and 027, which were associated with a significantly higher one-year overall mortality than that of patients with other types and controls. MLVA showed persistence of clones over prolonged periods of time.

**P629** Surveillance of nosocomial pneumonia and bloodstream infection in patients with acute leukaemia

C. Kiefer, D. Luft, R. Babikir, H. Bertz, W. Kern, A. Widmer, M. Dettenkofer* (Freiburg, DE; Basel, CH)

Objectives: We prospectively determined rates and incidence densities of nosocomial pneumonia and bloodstream infections (BSI) that occurred during neutropenia in patients with acute leukaemia (acute myeloid leukaemia [AML] and acute lymphatic leukaemia [ALL]) undergoing chemotherapy. The infection rates were compared to those of haematopoetic stem cell transplant (HSCT) patients.

Methods: CDC definitions for laboratory-confirmed bloodstream infection were used to define BSI, CDC adapted criteria to define pneumonia in neutropenic patients [for detailed information see http://www.nrz-hygiene.de/surveillance/onko.htm]. Data of six participating centres were entered into the ONKO-KISS_AL database, an extension established in 2005 of the well introduced ongoing multicentre infection surveillance project in haematopoetic stem cell transplant patients (ONKO-KISS: German Surveillance Programme for nosocomial infections in haematology patients) and analyzed.

Results: From 01/2005–06/2008 data on 1,012 consecutive patients (range 47 to 225 per centre), age ≥16 years, was provided with a mean duration of neutropenia of 16 days (range 2 to 106) resulting in a total of 16,262 neutropenic days. Mean duration of neutropenia was 17.3 days in AML patients and 12.3 days in ALL patients, respectively. The overall rate of BSI in acute leukaemia patients was 15% (in allogeneic HSCT patients: 16.5%), the BSI incidence density was 9.3 (8.6) per 1,000 neutropenic days. The overall pneumonia rate in acute leukaemia patients was 8.6% (in allogeneic HSCT patients: 11.4%), the pneumonia incidence density was 5.3 (6.0) per 1,000 neutropenic days for detailed results see table. BSI was more frequent in female patients (17.7% vs. 12.9% in males, incidence density 10.3 vs. 8.5). Pathogens isolated from blood cultures were Gram-positive cocci in 59.5% (most frequently: coagulase negative staphylococci 36%, enterococci 15%, streptococci 5%), Gram-negative rods in 37% (E. coli 20%, Pseudomonas aeruginosa 6%) and Candida spp. in 3.5%.

<table>
<thead>
<tr>
<th>Therapy groups</th>
<th>No. of patients (%)</th>
<th>Rate (%)</th>
<th>Incidence density</th>
<th>Rate (%)</th>
<th>Incidence density</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>ALL</td>
<td>AML</td>
<td>ALL</td>
<td>AML</td>
<td>ALL</td>
</tr>
<tr>
<td>All</td>
<td>768</td>
<td>244</td>
<td>16.0</td>
<td>11.9</td>
<td>9.3</td>
</tr>
<tr>
<td>Standard</td>
<td>345</td>
<td>161</td>
<td>15.1</td>
<td>11.2</td>
<td>8.3</td>
</tr>
<tr>
<td>Extended</td>
<td>100</td>
<td>47</td>
<td>18.0</td>
<td>4.3</td>
<td>13.2</td>
</tr>
<tr>
<td>Relapse &gt;60 years</td>
<td>291</td>
<td>32</td>
<td>28.0</td>
<td>na</td>
<td>12.9</td>
</tr>
</tbody>
</table>
| na: not applicable due to small group size.}
Incidence of infections in burn centres in France

C. Vinsonneau, F. Rucat, R. Le Floch, P. Amaud, M. Bertin-Maghit, H. Carsin, G. Perro, on behalf of SFETB

Objectives: Infections in burn patients are the leading cause of delayed death. To improve the prognosis of burns, prevention of infectious disease is mandatory, but large epidemiological study are lacking in our French population. This study aims to assess the incidence and characteristics of infections in this population.

Methods: Non-interventional study, epidemiological, longitudinal, conducted among 15 burn centres in France and held a period of three months from July to September 2006. Only new episodes of infection occurring during this period have been taken into account. Among the 784 patients admitted during the study period in participating centres, 348 had a burn centre stay strictly included within the range of the observation time.

Results: The characteristics of the whole population are (median, IQR): age 35.50 [16–52], sex ratio (M/F) 7/10, Total Burn Surface Area (TBSA) 10% [5–20]. 24% are younger than 15 years and 15% have a TBSA ≥ 30%. The overall incidence of infected patients is 19% (N=784) and 48.3% for burns over 30% (N=120). The overall incidence of infections is 32.7%. The density of incidence of infected cases is 27% of them are multi-resistant. Overall mortality is 5%.

Conclusions: Surveillance of nosocomial infections in burn centres is mandatory, but large epidemiological study are lacking in our French population. This study aims to assess the incidence and characteristics of infections in this population.
of stay (2 weeks 4.9 (3.8–6.2), 3 weeks 9.8 (7.5–12.8), 4 weeks 13.4 (10.5–17.0), ≥5 weeks 16.1 (12.7–20.3)).

Conclusions: The prevalence of hospital-acquired infections varies considerably between hospitals. These differences can partly be explained by differences in patient population.

General surveillance

P633 National network set-up for monitoring micro-organisms resistance to anti-infectives in a developing country: case of the Ivory Coast
N. Guessendi*, V. Gironon, D. Ouattara, B. Tiecouara, A. Achy-Brou, M. Dasso, on behalf of the ORMI-CI

Created in June 2000 under ONERBA (France) sponsorship, the Observatory of the Resistance of the Micro-organisms in Ivory Coast (ORMICI) set up a national network of sentinel laboratories for the monitoring of bacterial resistances to antibiotics. Since June 2006, ORMICI had become the National Reference Center for the monitoring of bacterial resistances in Ivory Coast.

Objectives:
- Standardising the methods of susceptibility tests and improving quality of information and the conditions of their collecting.
- Collecting information concerning micro-organisms resistances to anti-infectious evolution in Ivory Coast in order to analyze and diffuse them to the medical authorities, scientist societies and professionals of health.
- Training the prescribers and sensitising the population.
- Comparing the national data with those obtained in the other countries of the sub region.

Methods: The epidemiologic strategy of bacterial resistance monitoring is based on the choice of sentinel laboratories which can provide information in the Community and in Hospital. The activities were based on:
- network organisation and working,
- capacities reinforcement of the CNR and its network laboratories,
- network animation and management,
- strains stocks constitution.

CNR collects laboratories strains with epidemiologic information. Quality control is insured by the reference laboratory of antibiotics resistance study group of the International Network of the Pasteur Institutes.

Results: The network includes height public laboratories of various levels in the medical pyramid and nine private laboratories. A national campaign of sensitising to the right prescription of antibiotics was organised in collaboration with the General Mutual Insurance Company of Ivory Coast.

Several scientific workshops bringing together the professionals of the environment and those of human and animal health were organised. A regional course in susceptibility tests standardisation, financed by Pasteur Institute of Paris, has taken place in April 2008 in Abidjan. Information reports on the resistance levels were published. ARV resistance training intended for the prescribers has taken place in December 2008.

Conclusion: ORMICI was restructured in 2008 with the working out of a network animation and management, strains stocks constitution. CNR collects laboratories strains with epidemiologic information. Quality control is insured by the reference laboratory of antibiotics resistance study group of the International Network of the Pasteur Institutes.

P634 Inadequate drugs for the treatment of infections with Gram-positive pathogens using the EPICENTER Network data

Objectives: Surveillance data must help to identify drugs, which are obsolete for the empirical treatment of infections, because of resistance development. We analysed a one year period of the EPICENTER Network data to select drugs, which are useful for the treatment for infections with Gram positive pathogens.

Methods: At present 4 laboratories participate in the network using the automated BD PHOENIX system. The BD EPICENTER Data Management System is used for the evaluation of the data in the laboratory and for the transfer of the data to the concentrator for evaluation of the data with stratification by material, source, medical discipline, time, patient and others. Copy strains are excluded. Quality control is mandatory. Antibiotics with a rate of more than 35% resistance for a species were regarded as obsolete for empirical treatment.

Results: We analysed 1532 Enterococcus faecalis-, 638 Enterococcus faecium-, 3759 Staphylococcus aureus-, and 1718 Staphylococcus epidermidis-strains (see table). Blank fields indicate a drug–bug combination which is generally regarded as obsolete and was not tested or documented. R = intrinsic resistance. The isolates were from all specimen types. Analysing the data by specimen types, urine-, blood-, or pulmonary tract-isolates, the general outcome for infections with Gram positive pathogens.

Conclusion: Resistance %ages seen in the data of the EPICENTER Network indicate that an empirical treatment of Gram positive infections is even more difficult than with Gram negative ones and classical drugs like clindamycin and macrolides show doubtful results and should not be used without microbiological data of the patient. The only drugs which
still appear of some value are teicoplanin and vancomycin, however even with these drugs we must be prepared for the worst.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>E. faecalis</th>
<th>E. faecium</th>
<th>S. aureus</th>
<th>S. epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>100.0%</td>
<td>100.0%</td>
<td>5.00%</td>
<td>45.40%</td>
</tr>
<tr>
<td>Gentamicin-Syn</td>
<td>33.10%</td>
<td>43.90%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin-Syn</td>
<td>44.00%</td>
<td>77.20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic acid</td>
<td>0.90%</td>
<td>81.30%</td>
<td>32%</td>
<td>62.80%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.90%</td>
<td>87.10%</td>
<td>79.20%</td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td></td>
<td></td>
<td></td>
<td>32%</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td></td>
<td></td>
<td></td>
<td>73.00%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.60%</td>
<td>95.20%</td>
<td>32%</td>
<td>84.70%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>7.70%</td>
<td>91.70%</td>
<td>32.10%</td>
<td>73.40%</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>100.00%</td>
<td>100.00%</td>
<td>32.10%</td>
<td>73.40%</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0.00%</td>
<td>0.00%</td>
<td>76.40%</td>
<td>85.10%</td>
</tr>
<tr>
<td>Trimeth/ Sulfa</td>
<td>100.00%</td>
<td>100.00%</td>
<td>1.60%</td>
<td>33.60%</td>
</tr>
<tr>
<td>Fosfomycin w/G6P</td>
<td>0.40%</td>
<td>4.10%</td>
<td>0.80%</td>
<td>24.30%</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td></td>
<td></td>
<td>1.40%</td>
<td>21.00%</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.10%</td>
<td>3.30%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.20%</td>
<td>3.30%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>98.70%</td>
<td>95.90%</td>
<td>24.10%</td>
<td>49.40%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50.50%</td>
<td>90.40%</td>
<td>30.90%</td>
<td>71.80%</td>
</tr>
<tr>
<td>Mupirocin high level</td>
<td>1.40%</td>
<td></td>
<td></td>
<td>4.80%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>45.40%</td>
<td>86.30%</td>
<td>36.20%</td>
<td>61.20%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>39.70%</td>
<td>84.60%</td>
<td>35.70%</td>
<td>55.00%</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>36.00%</td>
<td>85.20%</td>
<td>30.80%</td>
<td>36.50%</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td></td>
<td></td>
<td>45.10%</td>
<td>51.10%</td>
</tr>
<tr>
<td>Rifampin</td>
<td></td>
<td></td>
<td>1.60%</td>
<td>8.10%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>75.10%</td>
<td>31.00%</td>
<td>4.80%</td>
<td>12.60%</td>
</tr>
</tbody>
</table>

Conclusions: Significant geographic variability in AG S was observed in the analysis of this large sample of GN pathogens with highest R rates in LA and APAC. Although the AG-R-mechanisms associated with these isolates were not assessed (companion abstract) it appears that a new generation of AG compounds would be a valuable therapeutic alternative to GEN, TOB and AMK for GN infections.

### Table

<table>
<thead>
<tr>
<th>Organism</th>
<th>%GEN R</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>3/2/9.3</td>
</tr>
<tr>
<td>KBs</td>
<td>3/7.9/7</td>
</tr>
<tr>
<td>EBS</td>
<td>5/8.7/7</td>
</tr>
<tr>
<td>CBS</td>
<td>3/2.3/8</td>
</tr>
<tr>
<td>SER</td>
<td>2/7.3/9</td>
</tr>
<tr>
<td>PM</td>
<td>6/4.3/7</td>
</tr>
<tr>
<td>IPF</td>
<td>11.5/12.4</td>
</tr>
<tr>
<td>PSA</td>
<td>14.7/12.6</td>
</tr>
<tr>
<td>ACB</td>
<td>31.6/42.7</td>
</tr>
</tbody>
</table>

*Isolates were non-S to GEN.

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D. Biedenbach, R. Jones*, G. Miller, E. Armstrong (North Liberty, San Francisco, US)

**Objectives:** To assess resistance (R) trends to aminoglycosides (AG) over ten years from a global sample of Gram-negative (GN) pathogens. This study determined the R rates of gentamicin (GEN), tobramycin (TOB) and amikacin (AMK) against nine common GN species groups from medical centres in North America (NA), Latin America (LA), Europe (EU) and Asia-Pacific (APAC).

**Methods:** Non-duplicate isolates from bloodstream and respiratory tract infections were collected from 38 countries between 1998–2000 (27,491 strains) and 2005–2007 (30,430 strains) via the SENTRY Program. Organisms included E. coli (EC), Klebsiella spp. (KBS), Enterobacter spp. (EBS), Citrobacter spp. (CBS), Serratia spp. (SER), P. mirabilis (PM), Indole + Proteae (IPP), P. aeruginosa (PSA) and Acinetobacter spp. (ACB). Susceptibility (S) testing was performed by two central monitoring laboratories using CLSI methods (M7-A7, M100-S18) and concurrent quality control testing.

**Results:** With the exception of KBS in EU, R to GEN increased in all regions for the three most prevalent Enterobacteriaceae (list first in the Table). Nearly all pathogens in NA showed increased GEN R rates (0.6–11.1%) during the last surveillance period (2005–2007), while significant variations were noted in other regions. The dramatic increase in GEN-R for nearly all species in APAC countries was due to strains sampled from countries not included in the earliest sample (e.g. India and Indonesia). Between 60–70% of the ACB were R to GEN in regions outside of NA, with the USA rate approaching 43% in the most recent years. R to all three AG agents for each of these pathogens was generally <1% among enteric pathogens from NA. However, much higher GEN/TOB/AMK-R rates were observed in other geographic areas for the most common Enterobacteriaceae, highest in LA 1.4–17.1% during both time periods. R to all three AG ranged from 3.1% (NA) to 25.3% (LA) for the PSA isolates collected during 2005–2007.

**Conclusions:** The analysis of this large sample of GN pathogens with highest R rates in LA and APAC. Although the AG-R-mechanisms associated with these isolates were not assessed (companion abstract) it appears that a new generation of AG compounds would be a valuable therapeutic alternative to GEN, TOB and AMK for GN infections.
Susceptibility of baseline *Pseudomonas aeruginosa* and *Acinetobacter baumannii* to doripenem and other antibiotics from six doripenem phase 3 clinical trials

K. Kamiya, R. Redman, I. Friedland, A. Quintana* (Raritan, US)

**Background:** In 6 worldwide phase 3 clinical trials of doripenem (DOR), the principal baseline non-fermenter isolates obtained were *P. aeruginosa* and *A. baumannii*. The distribution and susceptibilities of these pathogens to DOR and other antibiotics in regions where phase 3 DOR clinical trials occurred are reported.

**Methods:** The distribution of non-fermenters in 6 multinational trials of complicated intra-abdominal infections, complicated urinary tract infections including pyelonephritis, and nosocomial pneumonia (including ventilator-associated pneumonia) in North America (NA), South America (SA), Europe (EU), and Australia and South Africa (AU/SA) was calculated. The minimum inhibitory concentrations (MICs) for *P. aeruginosa* and *A. baumannii* isolates were generated utilising Clinical and Laboratory Standards Institute broth microdilution methods.

**Results:** The distribution of baseline non-fermenters were as follows: for *P. aeruginosa*, 27% (59/219) were from NA, 35% (77/219) were from SA, 35% (76/219) were from EU, and 3% (7/219) were from AU/SA. For *A. baumannii*, 29% (18/63) were from NA, 32% (20/63) were from SA, 29% (18/63) were from EU, and 11% (7/63) were from AU/SA. Overall, for all non-fermenters, DOR MIC50 and MIC90 were 0.5 and 16 mg/L, respectively, vs 1 and 32 mg/L for imipenem (IMI). For *P. aeruginosa*, DOR MIC50 and MIC90 were 0.5 and 4 mg/L, respectively, vs 1 and 16 mg/L for imipenem (IMI). For *P. aeruginosa*, DOR MIC50 and MIC90 were 1 and 32 mg/L vs 0.5 and 32 mg/L for IMI. When DOR MIC was 4 mg/L (including ventilator-associated pneumonia) in North America (NA), 29% (18/63) were from NA, 32% (20/63) were from SA, 29% (18/63) were from EU, and 11% (7/63) were from AU/SA. Overall, for all non-fermenters, DOR MIC50 and MIC90 were 0.5 and 16 mg/L, respectively, vs 1 and 32 mg/L for imipenem (IMI). For *P. aeruginosa*, DOR MIC50 and MIC90 were 0.5 and 4 mg/L, respectively, vs 1 and 16 mg/L for IMI. When DOR MIC was 4 mg/L (n = 12), 25% of *P. aeruginosa* had IMI MIC ≤ 4 mg/L. For *A. baumannii*, DOR MIC50 and MIC90 were 1 and 32 mg/L vs 0.5 and 32 mg/L for IMI, respectively.

**Conclusion:** In all regions, IMI MIC50 and MIC90 were generally double the DOR MIC50 and MIC90. Carbapenems had high MIC90 (32–64 mg/L) against *A. baumannii* across regions. While IMI MIC90 varied from 4–16 mg/L across regions, DOR MIC90 for *P. aeruginosa* varied little (2–4 mg/L).

Antimicrobial resistance surveillance in Korea in 2007: increasing prevalence of vancomycin-resistant *E. faecium*, cefotaxime- and cefoxitin-resistant *K. pneumoniae*, and imipenem-resistant *P. aeruginosa* and *Acinetobacter spp.


**Objectives:** Surveillance of antimicrobial resistance in Korea has been increasingly important with wide dissemination of bacteria resistant to clinically useful antimicrobial agents. Two surveillance methods have been used for the KONSAR (Korean Nationwide Surveillance of Antimicrobial Resistance) program: (1) analysis of routine test data generated by the participating hospitals; (2) collection and testing susceptibility of problem organisms by the coordinating laboratory. Aims of this study were to determine trends of resistance and emergence of new resistance.

**Methods:** Antimicrobial susceptibility test data generated in 2007 by 40 hospitals and one commercial laboratory (C-Lab) participating the KONSAR program were analyzed. The susceptibility was tested by either the CLSI disc diffusion method or commercial microbroth dilution methods.

Results: Of the 131,365 isolates, the ranks in decreasing order were: *E. coli*, *S. aureus*, *P. aeruginosa* (PAE), *K. pneumoniae* (KPN), coagulase-negative staphylococci (CNS), *E. faecalis*, *Acinetobacter* spp., (ACI), and *E. faecium* (EFM). Resistance rates at hospitals and at a C-Lab, which tested isolates mostly from small hospitals and clinics, are shown below. Comparison to the previous data in 2003 and 2005 showed that oxacillin-resistant (R) staphylococci, continued to be very prevalent at hospitals, whereas, it increased at the C-Lab. Increase of vancomycin- R EFM was significant at C-Lab. Further increases of cefotaxime-R *E. coli* (20%) and KPN (45%), and cefoxitin-R KPN (35%) at the C-Lab were new problems found. Imipenem-R rates of PAE and ACI were similar or even higher at the C-Lab.

**Conclusion:** Resistance of frequently isolated organisms continued to be prevalent or further increased. Recent efforts by the Health Insurance Review Agency Korea did not result in reduction of resistance, although significant reduction of inappropriate antimicrobials use had been reported. Further study is required to determine the genetic mechanisms of cephalosporin-R *E. coli* and KPN and of imipenem-R PAE and ACI.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Hospitals</th>
<th>Commercial Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2003</td>
<td>2005</td>
</tr>
<tr>
<td><em>Oxacillin-R S. aureus</em></td>
<td>68</td>
<td>59</td>
</tr>
<tr>
<td><em>Oxacillin-R CoNS</em></td>
<td>73</td>
<td>76</td>
</tr>
<tr>
<td><em>Vancomycin-R E. faecalis</em></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>Vancomycin-R E. faecium</em></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><em>Cefotaxime-R E. coli</em></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td><em>Cefotaxime-R K. pneumoniae</em></td>
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<td>25</td>
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<tr>
<td><em>Cefoxitin-R E. coli</em></td>
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<tr>
<td><em>Cefoxitin-R K. pneumoniae</em></td>
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<td>24</td>
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<tr>
<td><em>Imipenem-R P. aeruginosa</em></td>
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<tr>
<td><em>Imipenem-R Acinetobacter spp.</em></td>
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</tbody>
</table>

Susceptibility to levofloxacin-centred fluoroquinolones and other antibiotics of 19 species, 12,919 clinical isolates collected from 72 centres in Japan, 2007

K. Yamaguchi*, A. Ohno, Y. Ishii (Tokyo, JP)

**Objectives:** A detailed knowledge of the susceptibility to antimicrobial agents is important to facilitate the development of effective strategies to combat the growing problem of resistance. We have done a nationwide and longitudinal surveillance study to monitor resistance trends to levofloxacin-centred fluoroquinolones (FQs) in addition to other antimicrobial agents since 1994. In the present study, we analyzed the surveillance results from major bacterial species collected in Japan during 2007.

**Methods:** A total of 12,919 clinical isolates in 19 species were collected from 72 centres (the Levofloxacin Surveillance group) during 2007 in Japan. The activity of 30 antimicrobial agents against these clinical isolates was determined using the broth microdilution method recommended by the CLSI.

**Results:** The common respiratory pathogens, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Moraxella catarrhalis* showed a high “Susceptible” rate of 98% or more to FQs. The prevalence of macrolide resistance in *S. pyogenes* has been increasing from 2% to 25% during 1994–2007. The isolation rate of beta lactamase non-producing ampicillin-resistant *H. influenzae* was approximately eight times as large as those of western countries due to the high usage level of the third generation oral cephalins in Japan compared with the United State or Europe. Most strains of Enterobacteriaceae were also susceptible to FQs. The resistance rate of *Escherichia coli* to FQs has however been rapidly increasing from 2% to 25% since we started our surveillance in 1994. The FQ-resistant rate in methicillin-resistant *Staphylococcus aureus* (MRSA) was approximately 90% except for stafaxolin while FQs-resistance rate in methicillin-susceptible *S. aureus* was around 5%. In *Pseudomonas aeruginosa* clinical isolates, 25–30%
Molecular characterisation of fluoroquinolone resistance in *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* and *Escherichia coli* clinical isolates collected from 72 centres in Japan, 2007

A. Ohno*, Y. Izhii, K. Yamaguchi (Tokyo, JP)

**Objectives:** A detailed knowledge of the susceptibility to antimicrobial agents is important to facilitate the development of effective strategies to combat the growing problem of resistance. In this light, the molecular characterisation of fluoroquinolone resistance in *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* and *Escherichia coli* based on surveillance data conducted during 2007 in Japan to monitor the appearance of resistance to fluoroquinolones (FQs) was studied.

**Methods:** QDRD mutations of levofloxacin-resistant *Streptococcus pneumoniae* 474 isolates, *Streptococcus pyogenes* 8 isolates and *Haemophilus influenzae* 9 isolates, and 101 isolates of *Escherichia coli* with decreased susceptibility to fluoroquinolones were determined by PCR and direct DNA sequencing method.

**Results:** *S. pneumoniae* isolate with levofloxacin MIC 16/μg/mL possessed 3 points mutation. Two points mutation was shown in all with MIC 4/μg/mL and 8/μg/mL. In levofloxacin-susceptible isolates, 2 points mutation was detected in 4 of 384 isolates with MIC 1/μg/mL and 5 of 69 isolates with MIC 0.5/μg/mL.

*S. pyogenes* with MIC of 2/μg/mL were found in 8 of 509 isolates. Two points mutation was detected in 4 of 8 isolates and 3 of them showed MIC 16/μg/mL and 1 isolate was 8/μg/mL. One point mutation was also found in 2 isolates with 4/μg/mL and 1 isolate with 8/μg/mL. In *E. coli*, 8 of 9 isolates with MIC 1/μg/mL and 18 of 28 isolates with MIC 0.5/μg/mL had 2 points mutation or more. Most of rest isolates were all 1 point mutation. Two points mutation were also shown in 26 of 47 isolates with MIC 0.25/μg/mL and 12 of 17 isolates with MIC 0.12/μg/mL (other isolates did not have mutation). In *H. influenzae*, only 1 isolate showed levofloxacin-resistance (MIC 8/μg/mL) and this isolate had 3 points mutation. Two points mutation was detected in 1 isolates with MIC 2/μg/mL and 4 of 6 isolates with 0.5/μg/mL.

**Conclusion:** Quinolone-resistance was very few in *S. pneumoniae*, *S. pyogenes* and *H. influenzae* clinical isolates in 2007. Nevertheless, raise of MIC value was associated with the increase of number point in QDRRs. The rate of fluoroquinolone-resistance in *E. coli* has increased rapidly worldwide. Most resistant-strain have mutation of 4 positions or more (data not shown). Even in susceptible isolates with MIC ranged from 1 to 0.12/μg/mL, most isolates had at least 1 point mutation, and it may relate to the increase of resistant isolate.

Methods: Antimicrobial resistance of *P aeruginosa* animal isolates from house pets, farm and zoo animals (n = 54), with clinical signs of infection, previously characterised by AFLP fingerprinting, was evaluated by the disk diffusion method (CLSI) for 21 antimicrobial compounds, used in the treatment of human and veterinary infections. Biofilm formation was evaluated by Fluorescent In Situ Hybridisation (FISH), applied at different incubation times (24 h, 48 h, 72 h), and the relation between results was estimated using the Wilcoxon Signed Ranks Test.

Results: Antimicrobial resistance was similar among all isolates, showing high resistance to most drugs. None of the isolates was susceptible to all antimicrobials, while the majority possessed multi-resistance profile (97%). Resistance percentages were: amoxicillin/clavulanic acid, ampicillin, cephalaxin, cefotaxim, nalidixic acid, penicillin G, tetracycline, 100%; chloramphenicol, 97.96%; sulphame- toxazole/trimethoprim, 97.06%; streptomycin, 88.24%; enrofloxacin (ENR), 61.76%; carbenicillin (CAR), 67.65%; gentamicin (GEN), 67.06%; cephaperazone (CPF), 41.16%; amikacin, 33.5% drug-resistant 29.41%; ofloxacin, 23.53%; ciprofloxacin, 17.65%; ceftazidime, 11.76%; imipenem, tobramycin, 5.88%. About 18% of the isolates produced biofilm at 24 h. This percentage significantly increased with time. A significant relation (p < 0.05) was found between biofilm production at 72 h and antimicrobial resistance to most drugs (80.95%), with the exception for CAR, CFP, ENR and GEN.

Conclusion: Most of our isolates are multidrug resistant, while antimicrobial resistance increases with biofilm formation, especially for CAZ, CL, IPM, NA, PRL, TE and TOB. Monitoring antibiotic resistance of *P aeruginosa* from animal origin provides information on the antibiotic resistance prevalence outside human isolates. It contributes to a better understanding of drug resistance evolution and the potential resistance transmission to humans.

Surveying aminoglycoside resistance mechanisms: a tool for the development of neoglycosides

E. Armstrong*, D. Biedenbach, R. Jones, G. Miller (South San Francisco, North Liberty, US)

**Objectives:** To support the development of neoglycosides, the next generation of aminoglycosides (AGI) with an improved antibacterial spectrum, we conducted a survey of AG resistance mechanisms (AGRM) among selected clinical isolates. These data will be used to determine the spectrum of activity required of new compounds to overcome these mechanisms.

**Methods:** AGRM were surveyed using isolates of diverse geographic origin from the SENTRY 2005 and 2007 collections. Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from Europe and North/South America were chosen from the 2005 SENTRY Program collection based on established amikacin (A), gentamicin (G) and tobramycin (T) MICs. They were selected such that resistance rates to these AGIs in the test population matched those in the entire collection. MICs of 9 AGIs were determined for 406 strains and the resulting antibiogram was used to characterise the AGRM. Phenotypes for 6 common AGRM were confirmed by PCR (89 strains). Our study of the 2007 SENTRY Program collection focused on an extended-spectrum (ESR) Gram-negatives from Europe, North/South America, and Asia. MDR was defined by resistance to representatives of 3 drug classes; an aminoglycoside (A, G, or T), a fluoroquinolone (ciprofloxacin), and a third generation cephalosporin (ceftazidime or ceftriaxone). The strains selected for study were again representative of resistance rates to A, G and T within this population. MICs of 9 AGIs were determined for 302 strains and the resulting antibiogram used for AGRM characterisation. AGRM findings were compared to a published survey conducted between 1988 and 1993 (CID 1997;24:S46).

**Results:** The incidence of combined AGRM remained high and consisted of G and T modifying enzymes (AAC(3)-I, AAC(3)-II and ANT(2')-I) occurring as an aminoglycoside T-, netilmicin- (N) and A-modifying enzyme AAC(6')-I. The GTNA-resistance phenotype that results from these combinations also continues to occur as a result of permeability/exflux and now due to ribosomal methyltransferases (RMT). In all 3 studies the
objective: the cephamycin and carbapenem resistance mechanisms of recently isolated clinical Bacteroides strains whether there is a change with respect to the well-known resistance genes (cfxA, cfiA) and the mode of modulation of their expression by insertion sequence (IS) elements. We put a special attention to examination of strains displaying heterogenous resistance phenotypes for these two groups of antibiotics.

methods: for cefoxitin resistance 165 B. fragilis group strains were included that were isolated in Hungary in 2007–2008. For carbapenem resistance out of the 474 strains from the recent ESGARAB study investigating the antimicrobial susceptibility of Bacteroides strains, 12 isolates were examined that exhibited imipenem MICs ≥ 4 mg/l. Antibiotic susceptibilities were measured by agar dilution or Etests. The detection of resistance genes and regulatory elements were carried out by PCR. In interesting cases, regulatory regions were subjected to nucleotide sequencing.

results: Of the 165 Hungarian Bacteroides isolates from 2007–2008, 25 exhibited very high level resistance (≥ 256 mg/l) to cefoxitin or heterogenous resistance with resistant colonies at the 256 mg/l cefoxitin MIC value using Etests. Out of the 25 strains, 16 were cfxA-positive. Comparison of the regulatory region of cfxA genes of strains from a previous study that included cfxA-positive strains from a random collection with that of from this study demonstrated that the latter strains harboured mutations (mainly insertions of IS880 or deletions) in this region more frequently. Mutations involved disruption of an abortive phage-infection gene uniformly found in the random cfxA-positive strains. Out of the 12 Bacteroides strains with imipenem MICs ≥ 4 mg/l 6 were resistant (MIC ≥ 16 mg/l) of which 5 were cfiA-positive. Among these latter strains 4 harboured IS elements (2 IS56-like and 2 IS1187) in the regulatory regions of the cfiA genes and 1 did not but displayed a heterogenous resistance phenotype using Etests.

conclusion: The main type of activation of the cfiA gene was achieved by IS elements but a heteroresistant and a cfiA-negative strain also occurred. For cefoxitin heteroresistance mutations at the 3' end of the resistance element were very frequent. These mutations may be linked to the recently discovered amplification of the cfiA gene among Bacteroides strains and probably to their cefoxitin heteroresistant phenotype.
ciprofloxacin (CIP) was absent but DS to CIP was 10%, mainly S. Enteritidis from Spain. Cefotaxime (CTX) resistance was absent. DS to CTX was 2%. Gentamicin (G) resistance did not exceed 1%. In contrast, resistance to ampicillin, chloramphenicol, tetracycline (T), sulfisoxazole and trimethoprim/sulfamethoxazole amounted to 23, 18, 53, 41 and 15%. Totally, 512 C. jejuni (Ca = 258; P = 11; Ch = 243) and 1064 C. coli (Ca = 131; P = 752; Ch = 181) were recovered. Mean resistance (%) for C. jejuni (Ca; P; Ch) was: CIP 11, 9, 33; erythromycin (E) 0, 0, 0; G 0, 0, 0; nalidixic acid (NA) 18, 9, 35; T 29, 0, 87. Resistance to CIP for Ch isolates varied per country from 12 to 82%. DS to CIP did not exceed 1%; DS to E was 2%. In the case of C. coli, a much less frequent pathogen for humans, resistance (%) was: CIP 28, 34, 57; E 8, 33, 16; G 2, 5, 2; NA 34, 36, 57; T 57, 69, 80, respectively. E resistance was notably observed in porcine isolates from France and Spain.

Conclusions: This pan-EU survey, with uniform methodology, shows that clinical resistance among Salmonella from food animals to CIP or CTX, essential drugs for treating salmonellosis in humans, was zero. DS to both drugs was low. For most older drugs, notably higher rates of clinical resistance were assessed in Salmonella. Significantly, erythromycin resistance in C. jejuni, the major human pathogen of Campylobacter spp., was absent.

Antimicrobial susceptibility of commensal bacteria (Escherichia coli, Enterococcus) from cattle, pigs and chickens (2002–2004) recovered from 8 EU countries (EASSA programme)

Objectives: Antimicrobial susceptibility to human-use antibiotics was studied for commensal E. coli (Ec), and E. faecium and E. faecalis (Ent) from healthy food animals at slaughter across the EU.

Methods: Colon or caecal content was randomly collected at 4 abattoirs per country (n = 5/host). Each herd/flock was sampled once. Ec and Ent were isolated using standard methods. Susceptibility testing was done by agar dilution (CLSI, M31-A2) against 9 (Ec) and 5 (Ent) antibiotics in a central laboratory. Clinical resistance (CLSI, M100-S17) was assessed per drug/organism/country; decreased susceptibility (DS) was based on epidemiological cut-off values as defined by EFSA (2008).

Results: In total 3005 Ec were recovered (cattle (Ca) n = 991, pigs (P) n = 1015, chickens (Ch) n = 999). Mean resistance (%) for Ec for each respective animal host was: ampicillin (A) 7, 32, 51; cefepime 0, 0, 0; cefotaxime 0, 0, 0, 4; ciprofloxacin 1.3, 0.3, 6; chloramphenicol 3, 18, 14; colistin 0, 0.3, 9; gentamicin (G) 1.8, 1.5, 3.4; tetracycline 13, 68, 15; trimethoprim/sulfamethoxazole 6, 42, 47. DS was particularly apparent for ciprofloxacin (28% for Ch; 2–4% for Ca and P), whereas the corresponding figures for cefotaxime were 3 and 0.2–0.5%. In case of A and G, DS was negligible. For Ec, Italy (Ca), Spain (P), and Netherlands and Germany (Ch) generally showed the highest resistance; Denmark (P) showed the lowest. In total 1124 Ent isolates were recovered, comprising 975 E. faecium and 149 E. faecalis. All Ent were susceptible to linezolid. For E. faecium resistances to A, G and vancomycin (V) were 1–2%, whereas resistance to quinupristin/dalfopristin (Q/D) amounted to 31–33% for all 3 hosts. DS of E. faecium was only considerable for Q/D (31%). Though low prevalence of E. faecium limited conclusions, particularly in Ch (n = 14), resistance to A and V was absent; G resistance was low in Ca and P (0–8%), and intrinsic resistance to Q/D was noted (71 and 98%, respectively). DS was usually negligible.

Conclusions: This pan-EU survey, with standardised methods, shows that antimicrobial resistance among enteric commensal bacteria at slaughter was variable. For Ec, prevalence of resistance varied for older drugs and between countries but resistance to newer medically important antibiotics was absent or very low. With respect to Ent, quinupristin/dalfopristin resistance rates varied for E. faecium, but resistance was absent or very low for other drugs including linezolid and vancomycin.

Antibiotic susceptibility of invasive Neisseria meningitidis isolates from 1995 to 2008 in Sweden – the meningococcal population remains susceptible
S. Thalín Hedberg*, P. Olcén, H. Fredlund, M. Unemo (Orebro, SE)

Objectives: The aims of the present study were to describe the antibiotic susceptibility of all Swedish invasive Neisseria meningitidis isolates from 1995 to 2008 and to identify any longitudinal trends in the susceptibility and/or resistance.

Methods: All N. meningitidis isolates cultured in Sweden between 1995 and 2008 were included in the study (n = 717). The isolates were serogroup B (n = 391; 55%), C (n = 204), Y (n = 79), W-135 (n = 33), non-groupable (NG; n = 5), 29E (n = 2), A (n = 1), X (n = 1), and Z (n = 1). The minimum inhibitory concentrations (MICs) of penicillin G, pencillin V, cefotaxime, chloramphenicol, ciprofloxacin, rifampicin, and sulfadiazine were determined using the Etest method on Mueller-Hinton agar supplemented with 5% heated (“chocaloted”) horse blood. All isolates were also tested for beta-lactamase production.

Results: All isolates were fully susceptible to cefotaxime (MIC ≤ 0.12 mg/l) and ciprofloxacin (MIC ≤ 0.03 mg/l). No isolate was resistant to penicillin G (MIC > 1 mg/l) but in total 9% displayed reduced susceptibility (MIC > 0.094 mg/l). 59% of these isolates were serogroup B. The proportion of isolates with reduced susceptibility varied from 4% (in 1999) to 18% (in 2004). In 2008, only 5% of the isolates displayed reduced susceptibility. For penicillin V, during the years the susceptibility patterns were similar. However, in total 2% (0%-5% during the years) were resistant (MIC > 1 mg/l), and 50% of these were serogroup B. In 2008, no isolate was resistant. All isolates, except one serogroup B isolate from 2001 (MIC = 0.38 mg/l), were fully susceptible to rifampicin (MIC ≤ 0.25 mg/l). Concerning chloramphenicol, resistance (MIC > 4 mg/l) was observed in one isolate (serogroup B from 1996) and two isolates (one serogroup C from 2000 and one serogroup B from 2003) displayed reduced susceptibility (MIC > 2 mg/l). All the remaining isolates (99.6%) were fully susceptible. The percentage of sulfadiazine resistance varied between 52% and 81% over the years.

Conclusions: The Swedish population of invasive N. meningitidis isolates is still highly susceptible to the antibiotics used, both for prophylaxis and treatment. For penicillin G, in occasional years an increase of isolates with reduced susceptibility could be observed, but there was no obvious longitudinal trend towards a less susceptible population for penicillin G or any of the other antibiotics.

From concept to lab: preclinical vaccine development

The immunogenicity and protective immunity for a novel genetic vaccine against Mycobacterium tuberculosis with recombinant Plasmid expressing antigen Ag85A fused to cytokine L. Bao*, Y. Xie, W. Chen (Chengdu, CN)

Objective: BCG provides dissatisfaction protection against tuberculosis. Although DNA vaccines encoding Ag85A can induce strong immune response in vivo, the relatively low protective immunity may limit its practical use. The cytokine gene adjuvant can improve the efficacy of DNA vaccine. So we constructed the recombinant plasmid to express Ag85A fused to murine GM-CSF and detected its immunogenicity and protective immunity. Our objective is to improve the immunogenicity and protective efficacy of DNA vaccine against tuberculosis.

Methods: Ag85A gene was amplified from M. tuberculosis H37Rv and the cDNAs encoding GM-CSF were amplified from murine spleen-derived RNA. The recombinant plasmid pBK-GM/85A was constructed and the DNA vaccines were administered into mice to assess humoral and cellular responses. Serum antigen-specific antibodies were determined by ELISA. Lymphocyte proliferation assays and Cytokine assay were conducted for determination of cellular immune response.
The vaccinated mice were injected *M. tuberculosis* H37Rv and the mice's organs were homogenised to determine the number of CFUs.

**Results:** COS7 cells transfected with pBK-GM/85A and pBK-85A expressed 52 KD and 36KD protein respectively. The antibody titers of pBK-GM/85A were higher than that of pBK-85A in immunised mice. Both the recombint plasmid induced significantly higher lymphoproliferation than the control. The pBK-GM/85A was more potent than pBK-85A in the elevated stimulation index. The production of IFN-g with pBK-GM/85A was much higher than that with pBK-85A. Immunisation with pBK-GM/85A enhanced the amount of specific lysis compared to pBK-85A as the detection of CTL activity. As Protection against *Mycobacterium tuberculosis* challenge, vaccination with recombinant plasmid pBK-GM/85A or pBK-85A was capable of reducing significantly the number of CFU in the lungs compared with the control. pBK-GM/85A reduced the number of CFU more potently than pBK-85A (p < 0.05).

**Conclusion:** The protective efficacy for pBK-GM/85A was higher than that of pBK-85A in immunised mice. The results indicate that GM-CSF can potently enhance the immunogenicity and protective efficacy of *M. tuberculosis* DNA vaccine.

**[P660] IgA levels in lung and serum after oral immunisation with BCG encapsulated in alginate microspheres**

*M. Hosseini*, S. Ajdari, A. Adli Moghadam (Tehran, IR)

**Introduction:** BCG is the only available vaccine for prevention of tuberculosis. In tuberculosis induction of concurrent mucosal and systemic immunity protective against both pulmonary infection and systemic disease progression is desired. BCG is currently administered parenterally, which primarily stimulates systemic immune responses. Mucosal administration of vaccine offers the ability to trigger both mucosal and systemic immune responses.

**Materials and Methods:** In the present study, BALB/c mice were vaccinated orally with BCG encapsulated in alginate microspheres, then IgG and IgA levels in sera and lung homogenates, and DTH response were compared with those of mice vaccinated with free BCG by subcutaneous route.

**Results:** Mice immunised with encapsulated BCG and those immunised subcutaneously with BCG developed comparable DTH responses. IgA level in lung homogenate was significantly higher in the group immunised with encapsulated BCG than the group immunised with BCG subcutaneously. IgG level in lung homogenate was similar between two vaccinated groups. Serum IgG level was significantly higher in the group subcutaneously immunised than the group orally immunised with BCG, however immunisation with BCG either orally or subcutaneously produce similar level of IgA in serum.

**Conclusion:** Our data indicate that oral administration of BCG in alginate microspheres results in both systemic and mucosal immune responses.

**[P651] Protection of BALB/c mice against *Brucella abortus* 544 challenge by vaccination with combination of human serum albumin, L7/L12 recombinant fusion protein and lipopolysaccharide**

*I. Pakzad*, A. Rezaee, M.J. Rasaei, A. Zavaran hosseini, B. Tahbaraei (Ilam, Tehran, IR)

**Objectives:** The immunogenic *Brucella abortus* ribosomal protein L7/L12 and LPS are promising candidate antigens for the development of subunit vaccines against brucellosis. This study was aimed to evaluate the protection of combination of recombinant HASA-L7/L12 fusion protein with LPS in Balb/c mouse.

**Methods:** The amplified L7/L12 gene was cloned in pYHSA5 vector then pYHAS5-L7/L12 construct was transformed in *Saccharomyces cerevisiae* and expressed protein from supernatant was purified by affinity chromatography column. LPS was extracted by n-butanol, purified by ultracentrifugation. Balb/c mouses were immunised in 9 groups with PBS, HASA, tHSA-L7/L12, L7/L12, LPS, LPS-HASA, LPS-HASA-L7/L12, LPS-L7/L12, B. abortus S19. ELISA, LTT tests and challenging two weeks after last injection were carried out. Bacterial count of spleen of immunised Balb/c mouse was done four weeks after challenging with virulent strain *B. abortus* 544.

**Results:** In ELISA test the specific antibodies of HASA-L7/L12 exhibited a dominance of immunoglobulin IgG1 over G2a (IgG2a). LPS-HASA and tHSA-L7/L12-LPS produced a significantly higher antibody titer than LPS alone and L7/L12-LPS (P < 0.05). The predominant IgG subtype for LPS and L7/L12-LPS were IgG3. However, tHSA-L7/L12-LPS and LPS-HAS elicited predominantly IgG1 and IgG3 subtypes.

In addition, the tHSA-L7/L12 fusion protein and L7/L12 elicited a strong T-cell proliferative response upon restimulation in vitro with recombinant tHSA-L7/L12 and L7/L12, suggesting the induction of a cellular immunity response in vivo. However, There was no significant difference proliferative response in L/L12 and HSA-L7/L12 fusion protein (P > 0.05). The combination of tHSA-L7/L12 fusion protein with LPS and *B. abortus* S19 induce higher level of protection against challenge with the virulent strain *B. abortus* 544 in BALB/c mice than other groups (p < 0.005).

**Table 1. Protection of mice against challenge with *B. abortus* 544 after immunisation with various vaccines**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Log CFU/spleen, mean±SD</th>
<th>Log protection</th>
<th>p value</th>
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<tbody>
<tr>
<td>PBS</td>
<td>4.96±0.23</td>
<td>0.00</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HSA</td>
<td>4.86±0.26</td>
<td>0.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>L7/L12</td>
<td>3.75±0.27</td>
<td>1.2</td>
<td>&lt;0.05</td>
</tr>
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<td>HSA-LPS</td>
<td>3.91±1.16</td>
<td>1.0</td>
<td>&lt;0.05</td>
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<tr>
<td>LPS</td>
<td>4.27±1.02</td>
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<td>&lt;0.05</td>
</tr>
<tr>
<td>HSA-L7/L12-LPS</td>
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</tr>
<tr>
<td>L7/L12-LPS</td>
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<td>1.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S19</td>
<td>2.14±1.18</td>
<td>2.82</td>
<td>&lt;0.01</td>
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</tbody>
</table>

**Conclusions:** The combination of tHSA-L7/L12 fusion protein with LPS had higher protective ability than LPS and fusion protein distinctly.

**[P652] Evaluation of antibody response to group 2 outer membrane proteins of *Brucella abortus* s99 in animal model**


**Objective:** Outer membrane proteins of *Brucella* are considered as potential immunogens to develop a Brucellosis subunit vaccine. Although the antigen that dominates the humoral response in brucellosis is the lipopolysaccharide, outer membrane proteins would be characterised and evaluated since they are T-dependent antigens. In this study, Group 2 of OMPs or Porins of *Brucella abortus* s99 was initially extracted through an optimised method. Following the immunisation of animal models with extracted Porins, antibody response against these OMPs analyzed by ELISA method to determine anti-Brucella IgG titer in the sera of immunised animals.

**Methods:** Cells were suspended at 10mM tris buffer, and 1mg each of DNase and was added per 100ml. Samples were centrifuged twice to remove unbroken cells. Supernatants were submitted to ultracentrifugation to pellet the crude membranes. Detergent extraction of cytoplasmic membranes was performed by using sodium N-lauryl sarcosinate. Resultant insoluble material was dialyzed against tris buffer. In order to isolate the peptideoglycan from outer membrane proteins, lysozyme (1 mg/50 mg of membrane protein) was added. Samples were finally ultracentrifuged and supernatants were kept at 4°C. After biochemical evaluations of the extracted sample, animal models were immunised intramuscularly with *B. abortus* Porins and hyper immune sera of immunised animals collected. Animal models were immunised in three different patterns: (1) *B. abortus* Porins, (2) *B. abortus* Porins in combination with LPS, (3) *B. abortus* Porins in combination with Complete Freund’s adjuvant. Finally, titer of the elicited specific anti-Brucella IgG assayed by ELISA method in the sera of immunised animals and titers expressed in OD units.
ESBLs in hospitals, nursing homes and the community

P653 Serological evaluation of Brucella abortus S99 lipopolysaccharide extracted by an optimised method to be applied as a part of a candidate vaccine

Objective: Brucellosis is a globally found infectious disease and there is no licensed vaccine against human brucellosis. Brucellae can cause abortion in cattle and a debilitating fever (undulant fever) that may persist intermittently for years in humans. Lipopolysaccharide (LPS) is one of the main virulence factors and LPS-deficient strains have less virulence and intra-cellular survival potency. Wild type Brucellae mainly express smooth LPS (S-LPS) which is the main antigenic and immunogenic structure on the surface of smooth strains of this microorganism. A protective level of anti-Brucella IgG and IgM would be efficient to inhibit the primary infection and decrease the rate of infected Polymorphonuclears and macrophages.

Methods: Following the extraction of B. abortus S99 LPS by an optimised method based on hot phenol-water extraction, biological and biochemical evaluations of the extracted samples, animal models immunised intramuscularly with boosters in 14 and 28 days after the first injection. The animals were bled on the days 0 (before any immunisation and as the negative control), 14 (before the first booster injection), 28 (before the second booster injection) and 42 (two weeks after the second booster injection). The immune sera were separated, pooled and kept in −20°C.

Results: Sera of immunised animals have been reported positive by RBT as a result of B. abortus LPS immunogenicity which we extracted through our optimised method. The highest titer of anti-Brucella antibodies detected two weeks after the third immunisation (assayed by tube agglutination and rapid slide agglutination tests). All of the collected serum samples of immunised animals reacted specifically with the LPS of B. abortus and precipitation lines between B. abortus LPS and immune sera appeared after 30 minutes.

Conclusion: This modified extracted LPS of B. abortus S99 has efficiently promoted the synthesis of high levels of anti-Brucella antibodies. Furthermore, elicited antibodies reacted specifically with the extracted LPS (demonstrated by AGID). Potency of this structure to induce high titters of specific antibodies against Brucella suggests the possible application of this component as a part of a sub-unit or conjugated vaccine for human brucellosis.

P654 Construction of mutant pneumolysin pNK14 based DNA vaccine against Streptococcus pneumoniae
D. Bansal*, R. Verma, A. Kapil, J.C. Samanthuray, B.K. Das (New Delhi, IN)

Objectives: a. PCR amplification of wild Ply gene (1432 bp); b. Cloning of wild ply gene into pGEM-T Easy vector;


Methods: Wild type ply gene was PCR amplified and subjected to TA cloning using pGEM-T Easy Vector (3015 bp) and construct after sequence confirmation was subjected to deletion at A146AA to generate a nontoxic mutant construct. This mutant was sub-cloned in pET28a (Prokaryotic expression vector) for recombinant protein production and pNK14 for in-vitro expression evaluation in 293 HEK cell lines in tissue culture.

Results: a. PCR amplification of wild ply gene: Streptococcus pneumoniae Rx1 strain was amplified with primer having restriction enzyme sites for BamH1 and Acc65I. b. Cloning of wild ply gene into pGEM-T Easy vector: Amplified ply was cloned into p-GEMT Easy vector and construct obtained was confirmed by the single (EcoR1, PsI1, & Apo1) and double enzyme restriction digestions (BamH1 & Acc65I) and final confirmation by sequencing. c. Site directed mutagenesis (A146AA deletion): ply-pGEM-T construct containing mutation is denatured, primer annealed and pfu turbo polymerase extend and incorporate mutagenic primers resulting in nicked strands. DpnI enzyme digest the methylated non-mutated polymer and Agarose Gel Immunodiffusion (AGID).

Conclusion: Pneumolysin is produced by virtually all identified strains of S. pneumoniae. As pneumolysin is highly toxic, a nontoxic form of pneumolysin would be more desirable starting point in terms of vaccine production. Mutant Ply is prepared to generate non toxic ply which could be used for the DNA vaccine preparation. DNA Vaccine Mply-pPA
pNK14 DNA is based on ply gene fused with a signal peptide sequence tPA (pPA-ply) from pNK14 vector used for construct preparation which may induce predominantly Th2 type of protective immune response against Streptococcus pneumoniae when induced in BALB/c mice. Secreted pneumolysin may induce predominantly antibody mediated protection.

ESBLs in hospitals, nursing homes and the community


Epidemiology of extended-spectrum beta-lactamases in the Netherlands

Objectives: ISIS-AR was initiated medio 2007 in response to the widespread concerns about rising antimicrobial resistance in the
Netherlands and the lack of consistent long-term surveillance covering all clinical relevant pathogens. ISIS-AR is a combined effort of the RIVM-Centre for Infectious Disease Control, the Dutch Working Party on antibiotic policy and the Dutch Society for Medical Microbiology. It consists of a laboratory based surveillance system that collects the epidemiological and susceptibility data for each isolate present in the laboratory information system of a clinical microbiological laboratory (CML) on a monthly basis. In 2008, the first 8 CMLs were connected to ISIS-AR covering 34 pathogens. The aim of this study was to determine the prevalence and susceptibility patterns of Escherichia coli (EC) and Klebsiella pneumoniae (KP) isolates (intermediate) resistant (UR) to 3rd generation cephalosporins (CEPH3) in 1) nosocomial blood isolates, and 2) urine isolates from the hospital (HOSP), out-patient-departments (OPD), long-term care facilities (LTCF) and general practitioners (GP).

Methods: Data of the first isolate per species per patient collected from Jan-Oct 2008 were analysed.

Results: The prevalence of blood isolates 1/R to CEPH3 was 5.3% among 588 EC and 7.9% among 114 KP (2007 EARSS: Dutch data: EC 4.2%, KP 6.9%). An ESBL confirmation test was performed in 74.1% of the EC of which 78% were positive and 4.0% indeterminate. All of the 5 (56%) KP tested were positive. The prevalence of urine isolates UR to CEPH3 was 3.2% among 14,986 EC and 2.8% among 2,057 KP. For EC the prevalence was 4.2% in HOSP, 3.7% in OPD, 5.7% in LTCF, and 2.4% in GP. For KP the prevalence was 5.6%, 1.1%, 4.0% and 1.7% resp. ESBL positive EC were R/I to ciprofloxacin in 57%, aminoglycosides in 44%, cotrimoxazole in 71%. Co-resistance to these 3 antibiotics existed in 30%.

ESBL positive EC and KP urine isolates from GP were R/I to norfloxacin in 61%, cotrimoxazole in 70% and nitrofurantoin in 22%. Co-resistance to the first 2 antibiotics existed in 46% and all 3 in 7%.

Conclusions: This first analysis of the ISIS-AR database shows that (1) the Dutch ESBL prevalence rates are increasing, (2) ESBLs have entered the community and LTCFs, (3) oral treatment of urinary tract infections with ESBL resistant EC or KP in the community is entered the community and LTCFs, (3) oral treatment of urinary tract infections with ESBL positive versus ESBL negative isolates were as follows: for meropenem 97% vs 97%, respectively; for imipenem 91% vs 95% (NS); for etrapenem 43% vs 86% (p < 0.001); for cefotaxime 43% vs 93% (p < 0.001); for ciprofloxacin 20% vs 91% (p < 0.001); for tobramycin 20% vs 95% (p < 0.001); for amikacin 77% vs 97% (p < 0.001); for tigecyclin 40% vs 28% (NS) and for colistin 69% vs 69% (NS).

Conclusion: The prevalence of ESBL in Enterobacter spp. bloodculture isolates was 12.9%, showing that Enterobacter spp. are an important reservoir for ESBLs in the nosocomial setting. For optimal infection control, detection of ESBLs in Enterobacter spp. should be common practice in clinical microbiology laboratories. Phenotypic ESBL production in Enterobacter spp. is associated with increased resistance to etrapenem, ciprofloxacin, tobramycin, amikacin and cotrimoxazole.

[657] Acquisition of cephalosporin-resistant Enterobacteriaceae in relation to global exposure to antibiotic β-lactams in ten intensive care units of Paris metropolitan area

A. Thiébaut*, C. Berndère-Bauduin, G. Arlet, A. Andreemont, J.P. Sollet, D. Guilmot, B. Schlemmer on behalf of the ColoRea Group

Background: Nosocomial infections due to third-generation cephalosporin-resistant Enterobacteriaceae (CRE) have become a major public health threat, in particular in intensive care units (ICU). The influence of β-lactam exposure on CRE acquisition and selection still remains in debate.

Objectives: To investigate the dynamics of incident gut colonisation with CRE in combination with β-lactam use in ICU patients, we focused on the ecological link between global beta-lactam exposure of the population hospitalised in each unit and the incidence of CRE acquisition in previously “naïve” patients.

Methods: A prospective cohort study was conducted in 10 ICUs of Paris metropolitan area, in France, between November 2005 and February 2006 (ColoRea study). All patients admitted during the study period were followed-up until discharge. Rectal swabs were collected at admission, twice weekly thereafter, before β-lactam prescription and before discharge. Specimens were inoculated on agar supplemented with ceftazidime 2 mg/L and cefotaxime 2 mg/L. CRE were defined as isolates growing on the selective media showing decreased susceptibility to cephalosporines (diameter ≤17 mm, according to Clinical and Laboratory Standards Institute 2008) or to cefotaxime (diameter ≤22 nm), or producing extended-spectrum β-lactamase (Etest ESBL strip containing cefepime-clavulanic). Patients were informed about the study goals.

Results: In total, 917 patients provided 3,443 swab specimens (median, 3.2 per patient-week of follow-up). Of these, 109 (12%) were colonised with CRE at their first specimen collection (obtained within 48 hours of admission for 95%), including 48 with an ESBL-producing phenotype. Of the remaining 808 naïve patients, 115 (14%) acquired CRE during their follow-up (incidence rates ranging from 10 to 23 per 1000 patient-days in the 10 ICUs), including 39 with an ESBL-producing phenotype. A majority of patients (73%) had received β-lactams at least once, with defined daily doses ranging from 428 to 1003 per 1000 patient-days in the 10 ICUs. Correlations between antibiotic pressure at the ICU level and CRE acquisition rate among naïve patients were 0.27 for all antibiotics and CRE, 0.34 for β-lactam and all CRE, 0.40 for β-lactam and ESBL-producing CRE, and −0.14 for β-lactam and non-ESBL-producing CRE.

Conclusion: Global antibiotic exposure may play a role in CRE acquisition among ICU patients, which needs to be disentangled from individual exposure.
Cross-contamination does not explain the increase in ESBL-producing isolates in intensive care units in a French university hospital

V. Leflon-Guibout, F. Bert, S. Pease, C. Puigam-Burtz, N. Claudel, M.H. Nicolas-Chanoine* (Clichy, FR)

Objectives: To determine if the ESBL-producing enterobacteriaceae increase (attack rate, 3.7% in 2007 vs 7.7% in 2008) in 2 ICUs (33 beds) of our hospital was essentially related to cross-contamination.

Methods: For the 2 ICUs in which active surveillance of ESBL digestive carriage (rectal swab at admission and once a week: 1700 screenings/year) and precaution barriers have been implemented for several years, faecal or clinical ESBL-positive isolates (one isolate/species/patient) were identified (API System) and typed (ERIC-2 PCR method) from 1 May to 30 November 2008.

Results: Over the study period, the 72 ESBL-producing isolates from 57 patients (41 colonised and 16 infected), 27 (37.5%) were E. coli, 21 (29%) K. pneumoniae, 17 (25.5%) E. cloacae and 7 (10%) others. The rate of ESBL-positive isolates detected at admission was globally 37% (n = 27) with 60% for E. coli, 38% for K. pneumoniae and 12% for E. cloacae. Typing showed that 29 (64%) of the 45 remaining presumably ICU-acquired isolates had a unique ERIC-2 PCR profile ruling out cross-contamination. The 16 remaining isolates from 16 patients showed ERIC-2 PCR profiles shared with imported or another acquired isolates suggesting cross-contamination. Thus, 2 profiles were found for E. cloacae with 2 isolates each, 2 profiles for 9 K. pneumoniae with 5 and 4 isolates, respectively, 1 profile for 2 E. coli and 1 profile for 2 K. oxytoca, including an imported isolate. However, 2 profiles (1 for E. cloacae and 1 for K. pneumoniae) were found in the isolates from patients without overlapping ICU stay, excluding cross-contamination. Overall, 11 (19%) of the 57 patients with an ESBL-positive isolate acquired the isolate from another concomitantly hospitalised patient.

Conclusion: This study highlights the complex epidemiology of ESBL-producing isolates in our ICUs. ESBL-positive isolates of different species were present at the same time. One third of them was imported with an importation rate higher for E. coli than for the other species. Cross-contamination occurred but did not account for the majority of ESBL-positive isolated detected after 48h hospitalisation in our ICUs where precaution barriers are systematically applied to the patients with ESBL-positive isolates. A significant number of isolates that were not imported and not transmitted, emerged during ICU stay suggesting ESBL-positive isolate digestive carriage at such a low level that they were not detectable at admission.
**P662** In vitro activity of beta-lactam antibiotics against CTX-M-producing *E. coli*


**Objectives:** Multi-resistance is an increasing problem and it has been discussed that a beta-lactam antibiotic might be an option in the treatment of infections caused by multi-resistant ESBL-producing *E. coli* if the minimal inhibitory concentration (MIC) is low. Thus, the objective of this study was to investigate the activity of different beta-lactam antibiotics against CTX-M producing *Escherichia coli* in the County of Östergötland, Sweden.

**Method:** From 2002 to 2007, 95% of the clinical isolates of *E. coli* with ESBL-phenotype carried CTX-M-genes. One-hundred eighty-seven of these isolates were further analysed. PCR-amplification of CTX-M genes and DNA-sequencing of PCR-amplons were performed. MIC for amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, ceftazidime, cefotibuten, piperacillin-tazobactam and temocillin were determined using Etest (VITEK-2, Biomerieux, France) were performed by standard protocol. Patients and Methods: Observational and comparative study of a cohort of non-paediatric patients with ESBL admitted at a university affiliated hospital. Data collection from clinical records has been done according to a standard protocol. We analysed epidemiological, clinical, microbiological and laboratory data from January 2006 through May 2007. Patients with ESBL were identified by review of results of blood cultures from the hospital microbiology laboratory. *E. coli* isolation, identification and sensitivity test (VITEK-2; Biomerieux, France) were performed by standard criteria.

**Results:** 150 patients with *E. coli* bacteraemia were studied; prevalence of ESBL was 17% (n = 26; 12 female). Mean age was 57 years (range 14–94); 11 cases were considered nosocomial (53.8%) with a previous hospital stay of 12 days; 18 cases (69%) were patients in Medical Wards, 6 (23%) in Surgical Wards and 2 (7.7%) in ICU. An underlying condition was present in 22 patients (84.6%): cancer (11), obstructive uropathy (6), diabetes (5). Charlson comorbidity index was >3 in 15 cases (57.6%). Acute severity of illness at onset according to Winston criteria was “critical” in 11 patients, “poor” in 9 and “fair” in 6. Origin of ESBL was urinary in 9 cases (34.6%), abdominal in 5 (19%) and unknown in 12 (46%); 11 patients (42%) developed complications (renal failure, 10; shock, 7; respiratory distress, 3; and intravascular disseminated coagulation, 2). Empirical antibiotic treatment was wrong in 50%. Mean hospital stay was 20 days and mortality rate 64%.

**Conclusions:** Knowledge about risk factors and clinical aspects of ESBL is necessary to improve empirical treatments and decreased associated mortality and morbidity.

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**P661** In vitro activity of 13 antibiotics against *E. coli* from 8 major hospitals in Kuwait

G. Al Hashem, N.A. Al Sweih, W. Jamal, V.O. Rotimi* (Safat, KW)

Objective: The objective was to evaluate the antibiotic susceptibility of *Escherichia coli*, an important community and hospital pathogen, isolated from 8 Kuwait hospitals.

**Methods:** A total of 846 consecutively clinically significant strains of *E. coli* were studied during a one-year period. In vitro activity of 13 antibiotics against the isolates was determined by Etest. ESBL-production was assessed by ESBL-Etest method and confirmed by PCR technique. PCR amplicons positive for blaCTX-M were sequenced.

**Results:** About 69% of the *E. coli* isolates were highly non-susceptible to ampicillin with MIC90 of 256 μg/ml. Resistance to the 3rd generation cephalosporins ranged from 7.5% in Maternity hospital (MH) to 29% in Ibn Sina hospital (ISH); ciprofloxacin resistance rates ranged from 14% and 40%, respectively. Carbapenems and amikacin demonstrated excellent activities. Prevalence of ESBL-producing *E. coli* varied from hospital to hospital, with highest rate (32%) from ISH and lowest (4%) from Mubarak hospital and MH. MIC90 of cefotaxime, ceftazidime, cefepime and ceflapodox were >256, 64, >256 and >32 μg/ml, respectively for CTX-M-positive isolates versus 0.5, 1, 025 and 0.125 μg/ml for CTX-M-negative strains. Frequencies of CTX-M-positive isolates in cefotaxime MIC range of 1–2, 3–8, 9–16 and >16 μg/ml were 0, 4, 15 and 81%, respectively.

**Conclusion:** The prevalence of *E. coli* resistant to the 3rd generation cephalosporins and ciprofloxacin is at an unacceptable level. This is compounded by a high incidence of CTX-M ESBL-producing strains in almost all hospitals in Kuwait.

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**P664** Are CTX-M beta-lactamases associated with poorer clinical outcomes in bloodstream infections caused by ESBL-producing *E. coli*?


**Objectives:** The CTX-M-type ESBLs have recently undergone a rapid and global spread in Enterobacteriaceae. However, the clinical impact of
this epidemiologic change has not been well elucidated. We conducted a retrospective study to evaluate the differences of characteristics and clinical outcomes in patients with bloodstream infection caused by ESBL E. coli harbouring with or without CTX-M enzymes. 

Methods: From July 1, 2005 to June 30, 2007, patients older than 16 years with at least one positive blood culture of ESBL-producing E. coli were reviewed. ESBL production was screened and confirmed in accordance with CLSI standards. CTX-M β-lactamases were detected through multiplex PCR.

Results: During this 2-year study period, a total of 60 patients diagnosed of having ESBL E. coli bacteraemia were included in our analysis. CTX-M β-lactamases were detected in 41 blood culture isolates through multiplex PCR. Univariate analysis showed those subjects harbouring CTX-M enzymes were less frequently associated with renal failure (p = 0.01), ICU hospitalisation (p = 0.04), carriage of nasogastric tubes (p = 0.005) and central venous catheters (p = 0.025). In multiple analyses, renal failure was the only independent risk factor of acquiring non-CTX-M type ESBLs. The most commonly identified primary infection site was urinary tract in both groups. Although the early and late mortality rates did not differ significantly in these two groups of patients, those harbouring CTX-M enzymes seemed to have lower disease severity in terms of requirement of sequential ventilation and development of septic shock at onset of bacteraemia. Five patients were identified as having strictly community-acquired infection and all of them had CTX-M β-lactamases detected in the blood isolates. All of these 5 patients survived despite 2 of them had received inadequate empirical antimicrobial treatment.

Conclusion: CTX-M enzymes have emerged as the predominant type of ESBLs in E. coli bloodstream isolates in many parts of the world including Taiwan. Comparisons among bloodstream infections caused by ESBL-producing E. coli showed CTX-M β-lactamases were not associated with poorer clinical outcomes.

**P665 Multidrug resistance of Klebsiella pneumoniae isolates in a teaching hospital**  

Objective: Klebsiella pneumoniae is emerging as an important nosocomial pathogen due to rapidly increasing resistance to all currently available antibiotics. The aim of this study was to define the susceptibility profile of multidrug resistant (MDR) K. pneumoniae nosocomial isolates to antibiotics, including tigecycline (TIG), during the last three years in Patras University Hospital.

Methods: From September 2005 to October 2008, a total of 216 K. pneumoniae isolates were collected from inpatients hospitalised in ICU (125), in Internal Medicine units (48) and in Surgical Wards (43), one isolate per patient. Isolates were recovered from cultures of clinically significant specimens (102 blood, 76 pus, 24 urine and 14 BAL). Identification was performed by biochemical tests. Antimicrobial susceptibility was carried out by disk diffusion method, according to CLSI criteria, for amoxicillin-clavulanic acid (AMC), cefotaxin (FOX), ceftazidime (CAZ), imipenem (IMP), aztreonam (AZT), gentamicin (GM), netilmicin (NET), amikacin (AN), ciprofloxacin (CIP) and by E-Test strips (AB Biodisk) for TIG. MIC breakpoint of susceptibility to TIG is equal or less than 2 μg/mL. IMP-resistant isolates were examined by E-Test (AB Biodisk) for detection of metallo-beta-lactamases (MBL). In addition the genes encoding MBL (vm) and imp were detected by PCR following by sequencing.

Results: Resistance rate to lactams was as high as 99%, 94%, 100%, 97%, 94% and 79% to AMC, FOX, CRO, CAZ, AZT and IMP, respectively, 87% to AN and CIP, and 91% to NET and 61% to GM. Among 216 MDR isolates, 49% were resistant to all aforementioned antibiotics. Among IMP-resistant strains, 150 (88%) were MBL (+) according to E-Test results, whereas, in 164 (96%) were found to carry the vim-1 gene. Among MDR isolates 90% were susceptible to TIG (MIC 0.25–2). On beginning the study, 5% of 28 MDR isolates were intermediate sensitive to TIG (MIC 3), 7% of 77 the next year and 12% of 111 on 2008. Two resistant strains to TIG with MIC 19 were found during the last month of the study.

Conclusions: A total of 107 (49%) MDR K. pneumoniae were resistant to all commonly used antimicrobials. The presence of vim-1 gene (96%) in MDR isolates make IMP not useful in empiric therapy. The only active agent towards such strains remains TIG (90%), although 9% were immediately sensitive isolates according to our results. However TIG must cautiously be used, since resistant strains have already emerged.

**P666 Escherichia coli and other Enterobacteriaceae. Antimicrobial susceptibility trends in a four-year prospective surveillance hospital monitoring**  
R. Manfredi*, A. Nanetti (Bologna, IT)

Introduction: The changed rate of drug resistance among Enterobacteriaceae is a relevant issue, especially in hospital facilities. A prospective microbiological surveillance based on a continued monitoring of in vitro antimicrobial susceptibility rates, is ongoing at our General Hospital, since the year 2004.

Materials and Methods: The temporal variations of in vitro antimicrobial sensitivity trends were updated quarterly for all suitable Enterobacteriaceae strains, followed from year 2004 to year 2007. The same pathogen cultured more than once from the same patient within one month, has been considered one time only.

Results: Among overall Escherichia coli isolates (4,413 strains tested on the whole), imipenem and colistin maintained a full (100%) in vitro activity, followed by amikacin (97.3–99.5% of tested strains), nitrofurantoin (98.2–94.8%), piperacillin-tazobactam (89.0–93–9%), gentamicin (81.9–89.4%), cefazidime (78.3–89.5%), cefotaxime (78.0–89.8%), and ciprofloxacin (63.8–73.9%). When considering Enterobacteriaceae other than Escherichia coli, imipenem and colistin remained 100% active, followed by amikacin (94.9–97%–2%), piperacillin-tazobactam (78.4–86%), Cotrimoxazole (72.1–78.0%), gentamicin (74.0–77.3%), norfloxacin (66.4–76.2%), cefazidime (62.7–69–7%), and cefotaxime (62.3–69.0%). The emerging spread of enlarged-spectrum beta-lactamase production significantly reduced the activity of third-generation cephalosporins over time (from a mean of 89.7% of susceptible Escherichia coli strains in the year 2004, to 78.1% in the year 2007; p < 0.001; and from a mean of 69.7% of sensitive Enterobacteriaceae strains in the year 2004, to 62.4% in the year 2007; p < 0.03). Also fluoroquinolones and protected beta-lactams suffered from a drop of their in vitro sensitivity rates (p < 0.02 to p < 0.005).

Conclusions: A long-term prospective bacteriological monitoring of antimicrobial susceptibility rates of relevant hospital-related microorganisms like Enterobacteriaceae is of paramount importance, to plan antibiotic treatment and prophylaxis schedules, in common, local clinical settings. Despite a maintained activity of carbapenems the old colistin, a significant trend toward increased resistance rates was found over a four-year observation period, with extended-spectrum beta-lactamase secretion playing a major role.

**P667 Temporary changes in the frequency of extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumoniae in Colombia, 2002–2008**  
A. Meneses, G. Buitrago, J. Cortes*, A. Leal, J. Castillo, C. Alarcèz (Bogota, CO)

Objective: To describe the trend in time of the frequency of E. coli and K. pneumoniae isolates with extended lactamase beta (ESBL) production in colombian third level hospitals.

Methods: A time series analysis of E. coli and K. pneumoniae ESBL producers, obtained in clinical samples from hospitalised patients in 9 third level hospitals in 2 cities in Colombia (Bogota and Ibague) between January 2002 and June 2008. All the isolates had an ESBL confirmed by use of automated microbiological Vitek system (Lyon, Biomérieux). Data was systematised with Whonet 5.4®. Isolates were characterised according to ward type (ICU vs. non ICU) and sample type. Monthly
Results: Model simulation results fit well with observed prevalence rates in recent years. Inside and outside the hospital the prevalence of ESBL+ carriage is for 2008 estimated to be 7% and 2.5%, respectively, for KP and 4.5% and 0.3%, respectively, for EC. Without changes in infection control measures, an equilibrium prevalence will be reached after 7 years. Equilibrium prevalence in ICUs will be 11% for patients colonised with EC+ and 8% for those colonised with both “KP+ and EC+”, respectively. The equilibrium prevalence will be highest (19%) for both ESBL+ species in the high risk wards, because of the highest readmission rates of chronically ill patients. Conjugation between EC and KP contributes to 14% of the acquisition of KP+.

Conclusions: A multi-compartment deterministic model fitted to the observed increase in ESBL prevalence in the last 10 years, predicts that, in the absence of interventions, the number of patients per year with KP+ and EC+ will increase 116% and 67%, respectively, within the next 7 years.

P669 Faecal colonisation with extended-spectrum β-lactamase producing Enterobacteriaceae among patients in nine Swedish nursing homes


Objectives: Extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL-EB) have increased during the last years also in low-prevalence countries like Sweden, and mandatory laboratory reporting has been introduced on a national level. Little is known about the population prevalence of ESBL-EB in Sweden, and information is also largely lacking on source of the isolates (community vs hospital). The objectives of this study was to investigate the prevalence of ESBL-EB among the total population of elderly patients living in nursing homes in Solna County, north of Stockholm, Sweden.

Methods: Faecal swabs from 495 elderly living in nine nursing homes were collected during a five week period in October and November 2008 and sent for cultivation at Karolinska University Laboratory. Faecal swabs were cultured on selective chromID ESBL plates (bioMérieux). Species determination was performed with in-house biochemical tests and presence of ESBL was investigated with disks (Becton Dickinson) and E-tests (bioMérieux). ESBL-producing isolates were epidemiologically characterised using pulsed-field gel electrophoresis (PFGE) when several cases were detected in the same nursing homes. A previously described real-time probe based PCR was used for typing of blaCTX-M to the phylogenetic subgroups.

Results: Fifteen of the 495 elderly living in seven of the nine nursing homes were ESBL positive (3%). The majority of the elderly were colonised with Escherichia coli (14/15), followed by Klebsiella pneumoniae that was found in two patients, one of which was co-colonised with E. coli, K. pneumoniae and Citrobacter koseri. PFGE patterns on Xbal digested DNA was analysed for eleven E. coli isolates from elderly living in four nursing homes with several cases. In two of the homes a close correlation was found between the isolates, indicating transmission between patients. PCR typing of blaCTX-M among E. coli showed that 11 belonged to CTX-M-1 subgroup, one to CTX-M-9 subgroup, and two isolates were blaCTX-M-negative. Among K. pneumoniae and C. koseri all isolates were blaCTX-M-negative.

Conclusion: To our knowledge this is the first population based prevalence study of ESBL-carriage in Scandinavia. In the study population 3% were colonised with ESBL-producing Enterobacteriaceae. Only a few examples of possible local transmission were documented.

P670 Epidemiology and clinical features of infections caused by extended-spectrum β-lactamase-producing Gram-negative urinary pathogens in non-hospitalised patients

A. Vanegas *, P. Merino, J. Pérez, FJ. Candel, JJ. Picazo (Madrid, ES)

Objective: Infections due to extended-spectrum β-lactamase (ESBL)-producing Gram-negative urinary pathogens in nonhospitalised
ESBLs in hospitals, nursing homes and the community

S159

patients and their clinical relevance have constantly emerged during the past years. Therefore, the epidemiology data and antimicrobial susceptibility tests would contribute to enhance knowledge from these pathogens. The aim of this study was to describe the epidemiology and clinical features of patients with urinary tract infection caused by Enterobacteriaceae ESBL and its antimicrobial susceptibility.

Methods: A retrospective descriptive study was carried out. Enterobacteriaceae ESBL strains isolated from patients with urinary tract infections (UTI) in outpatient clinics from primary care services and the emergency department from Hospital Clínico San Carlos were used as target group between January of 2007 and August of 2008. Clinical protocols and epidemiology data from patient’s medical history were collected.

Results: A total of 185 ESBL strains were isolated from patients with UTI. 140 (75%) Escherichia coli and 39 (21%) Klebsiella pneumoniae. 62% were females and 37% males. The median age of patients with E. coli and K. pneumoniae was 69 and 60 years respectively (P <0.001). 50% were from outpatients and 33% from emergency room patients. From 60 patients (32%) without a previous hospital admission 55 (39%) were diagnosed with UTI occasionated by E. coli and 4 (10%) by K. pneumoniae (P <0.001). The most frequent features associated to infection by E. coli and K. pneumoniae were to use betalactamics or quinolones antibiotics in the previous 3 months 55% and 66% (P=0.27), comorbidity 50% and 74% (P=0.10), recurrent urinary tract infections 44% and 53% (P=0.36), urinary abnormalities 36% and 56% (P=0.28), hospitalisation in the last 3 months 33% and 66% (P <0.001), and renal transplant 5% and 38% (P <0.001) respectively. Resistance to quinolones in E. Coli and K. pneumoniae were 54% and 61% respectively, and to aminoglycosides 39% and 39%. Fosfomycin-resistance were detected in 17% and 38% respectively.

Conclusions: Elderly patients, comorbility, renal transplant, prior use to betalactamics or quinolones and previous patient hospitalisation were the most frequent characteristics associated with UTI. Antibiotic resistance such in quinolones and aminoglycosides has to be considered in advance as well as the epidemiological data from patients before establishing an accurate treatment.

P671 Community-onset urinary tract infections caused by ESBL-producing Enterobacteriaceae


Objectives: ESBL-producing Enterobacteriaceae (ESBLE), especially E. coli, are increasingly identified in community patients mainly with urinary tract infections (UTI). We defined the prevalence of community-onset versus hospital-acquired UTI caused by ESBLE. The clinical data of patients with community onset UTI caused by ESBLE were reviewed.

Methods: A total of 1169 single-patient isolates, recovered from urine cultures of 776 hospitalised patients and 393 outpatients during 2008, were studied. The bacterial species studied were E. coli, K. pneumoniae and P. mirabilis. The identification, MIC determination and screening for ESBLs were performed by the Vitek 2 compact automated system. CLSI approved confirmatory test (combined disk test) was also applied. Clinical isolates co-expressing carbapenemase phenotypes were excluded. All community strains were tested by PCR. The demographic and clinical characteristics of patients were evaluated using medical records.

Results: 83 patients (7.1%) harboured uropathogenic ESBLE. Species distribution was: E. coli 66, 72%; K. pneumoniae 14, 11.7%; P. mirabilis 3, 2.3%. Hospital-acquired infections were considered in 24 patients. Healthcare-associated infections were identified in 13 patients (outpatients under haemodialysis or chemotherapy, surgery, prior hospitalisation the past one month). The criteria for community-onset infection were met in 46 patients (55.4%). Female gender was prevalent (33/46). Mean age was 74.9 y (range 27–91 y). Regarding E. coli isolates 36/66 (54.5%) were characterised as community-acquired and resistant rates to quinolones and cotrimoxazole were 79% and 67% respectively, whereas all ESBL-producing P. mirabilis isolates were hospital-acquired. Predominance of community-onset infections was noted for K. pneumoniae (10/14). The rate of ESBLE among patients with community-onset UTI was 7.4% (46/624). E. coli 7% (36/510) and K. pneumoniae 18.5% (10/54). The proportion of ESBLE among hospital and healthcare-associated UTI was 7.4% (37/499). PCR identified 33/36 community E. coli isolates as carrying blaCTX-M gene.

Conclusions: The rate of uropathogens expressing ESBL was similar in community and hospital-acquired strains. This indicates a change in the epidemiology of ESBLE, with a rather high proportion of community isolates to express ESBL, especially CTX-M. It is suggested that empirical administration of beta-lactams for community UTIs should be done with caution in high risk patients.

P672 Emergence of community-onset extended-spectrum β-lactamases-producing Escherichia coli in acute pyelonephritis in Korean hospitals

J.W. Chung*, S.H. Choi, S.M. Moon, M.S. Lee (Seoul, KR)

Background: Extended-spectrum β-lactamases (ESBLs) produced by Gram-negative bacteria is a growing threat in hospital-acquired infections worldwide. Recently, a few studies have reported the dissemination of ESBL producers throughout the community. We report several cases of community-onset ESBL-producing Escherichia coli in acute pyelonephritis (APN) from the Korean hospitals.

Methods: The cases of community-onset APN caused by ESBL producers were collected from 3 secondary or tertiary care hospitals in Seoul, Korea. They were regarded as community-onset when they presented community-acquired infections (developed within 48 hours of hospital admission), no hospitalisation in the last one year, no transfer from other hospitals, no stay in nursing home, no urinary device and no antimicrobial treatment in the previous 6 months. Species identification and antibiotic susceptibility test were performed with VITEK II automated system and ESBL production confirmed by double disk synergy test. Furthermore, characterisation of ESBL encoding genes is being performed and the results will be presented later.

Results: From January 2007 to December 2008, there were 8 cases of community-onset APN, caused by ESBL-producing E. coli, in the hospitals. All cases developed in women with median age of 61 years (ranged from 36 to 79 years). Any serious underlying disease or condition was not found, except diabetes mellitus in two patients. Antimicrobial resistance to amikacin (none of 8), gentamicin (2 of 8), trimethoprim-sulfamethoxazole (2 of 8) and tobramycin (1 of 8) were not observed frequently. However, resistance to ciprofloxacin was observed in 4 of 8 cases (50%), one of whom died during inappropriate empirical therapy with ciprofloxacin and ceftriaxone.

Conclusion: Our study suggests that APN caused by community-onset ESBL-producing E. coli may develop in women without serious underlying diseases or risk factors for hospital acquisition of ESBL-producing Gram-negative bacteria. The emergence of community-onset infections caused by ESBL-producing pathogens will make the empirical therapy difficult in Korea, where ciprofloxacin or ceftriaxone is recommended for empirical therapy for APN. Close observation will be needed for the emergence of ESBL-producing organism in the community.

P673 Faecal carriage and household transmission of CTX-M producing Escherichia coli

WU. Lo1*, PL. Ho, K.H. Chow (Hong Kong, HK)

Background: Escherichia coli that produce CTX-M type extended-spectrum β-lactamases (ESBLs) have emerged as significant pathogens worldwide and increasingly found from patients with community-onset infections.

Objective: To investigate the epidemiology of faecal colonisation by CTX-M producing E. coli among thirty families and to assess the extent of transmission within family members.
Methods: Selective media (MacConkey agar with cefalexin or cefotaxime at 2 mg/L) were used for screening faecal samples from the family members. Colonies of E. coli were picked for susceptibility test and molecular studies by PCR, sequencing and PFGE.

Results: CTX-M-producing E. coli isolates were found in at least one member in 20 (67%) of the 30 families and 43 (34%) of 127 participants. Faecal prevalence was similar among children and adults (37% vs. 33%, respectively). Six different CTX-M enzymes were found. CTX-M-14 accounted for 61% of all CTX-M producers. Of the 20 families with CTX-M type ESBL carriers, thirteen families had >1 carriers and 7 families had at least 2 members carrying the same CTX-M allele. However, pulse field gel electrophoresis (PFGE) showed that most CTX-M producers within families were not clonally related.

Conclusion: Our result demonstrates that the faecal carriage of CTX-M-producing E. coli is strikingly high in Hong Kong community, in comparison with other areas. Nonetheless, household transmission does not seem to play a major role in their dissemination.

Molecular fingerprinting of faecal isolates of E. coli with and without ESBL-phenotype in patients with intra-abdominal infections

Objectives: To investigate clonal relatedness of faecal isolates of E. coli with and without ESBL-phenotype from 9 patients with intra abdominal infections, sampled before, during and after antibiotic treatment.

Method: From the 9 patients 76 faecal isolates of E. coli with different antibiograms, with and without ESBL-phenotype were isolated. All isolates with different antibiograms at each sampling time were further analysed. MIC-determination of the isolates were made for 16 antibiotics representing common prophylactic agents, treatment options and last line treatment of multi drug resistant isolates. The isolates were subjected to PCR-amplification and DNA-sequencing for detection of genes belonging to the CTX-M and SHV-families. All isolates were fingerprinted four times using a semi automated approach and the genes belonging to the CTX-M and SHV-families. In Italy, a large multicentre, prospective, cohort study is ongoing to assess the molecular epidemiology, clinical impact, treatment outcome and risk factors for infections caused by ESBL-producing Enterobacteriaceae. We report the preliminary results from one of the study centres, where a major evolution of the ESBL epidemiology has been observed in the past few years.

Methods: ESBL screening was carried out using modified cefotaxime and cefazidime breakpoints, as recommended by the CLSI. ESBL production was confirmed using the combination disk test, based on CLSI methodology. ESBL determinants were investigated by PCR and sequencing. Patients with ESBL infections and matched controls were prospectively enrolled in the study. Data on risk factors, therapy and outcome were collected in an electronic database.

Results: 65 cases of infections caused by ESBL-producers observed at Florence University Hospital were studied during a 12-month period (July 2007-June 2008). The most prevalent ESBL-positive species was Escherichia coli (77%), followed by Klebsiella pneumoniae (12%). CTX-M-type enzymes accounted for 74% of the ESBL producers. The ESBL epidemiology was found to be profoundly changed in comparison with that observed in 2003, in the same centre, during a nationwide survey (E. coli and CTX-M-type enzymes accounted for 15% and 1% of the ESBL producers, respectively). Isolates were mostly from urinary and lower respiratory tract infections. Almost half (43%) of infections were from internal medicine/geriatrics wards. Previous hospitalisation and presence of urinary catheter were associated with infection with ESBLs producing Enterobacteriaceae. Empirical treatment resulted appropriate according to in-vitro susceptibility testing in 69% of the cases. Inappropriate therapy was mainly (88%) based on fluoroquinolones.

Conclusion: A recent and massive dissemination of CTX-M-producing E. coli modified remarkably the ESBL epidemiology in this hospital setting. Similar strains now pose a major clinical challenge.

Acknowledgements: Study supported by a research grant from Merck Sharp & Dohme.

Urinary tract infections and sexually transmitted diseases

Multiresistant urinary tract isolates of Escherichia coli: is it an issue in the empiric management of acute uncomplicated cystitis?

Objectives: To assess the prevalence of multiresistance (multiR) among E. coli strains isolated from patients with community-acquired urinary tract infections (UTI).

Methods: Escherichia coli strains were isolated from outpatients >16ys referred for urine culture. Resistance (%)R was defined by disk diffusion according to CLSI 2006. A questionnaire accompanied each sample, in order to differentiate acute uncomplicated cystitis cases (AUC). MultiR was defined as R to 3 or more agents among ampicillin (AMP), cephalothin (CEP), nitrofurantoin (FUR), cotrimoxazole (COT) and nalidixic acid (NAL). R to amoxicillin-clavulanate (AMC), cefuroxime axetil (CXM), mecillinam (MEC), fosfomycin (FOS) and ciprofloxacin (CIP) was also defined. Risk factors for multiR strains isolation were processed by univariate analysis and parameters with P <0.1 were entered in a multivariate logistic regression stepwise model. Odds ratio (OR) and 95% confidence intervals (95%CI) were calculated. A P value <0.05 was considered as statistically significant.

Results: From Feb 2005-Mar 2006 and a total of 1545 E. coli strains, 731 cases of AUC were identified. %R rates in AUC were: AMP 25.5,
The effect of sewage treatment on antibiotic resistance of enterobacteria

M. Österblad*, A. Hakanen, J. Jalava, P. Huocinen (Turku, FI)

Objectives: Wastewater can potentially spread resistant bacteria. Most of the bacteria are removed from the purified wastewater, but some are still released. Studies on the effect of purification on resistance are conflicting. Treatment methods have also changed. We sampled sewage plants of different sizes, all using biological-chemical treatment. Resistance levels of Enterobacteriaceae in raw sewage and efflux were compared.

Methods: Wastewater samples were collected in Southwestern Finland in December 2001 – June 2002. The samples were suitably diluted and plated on duplicate plates: Rambach agar without antibiotics, and with 100 mg/l trimethoprim, 8 mg/l tetracycline, 25 mg/l streptomycin, 20 mg/l nalidixic acid, and 1 mg/l ciprofloxacin. All blue colonies were counted on plates with a suitable density of growth. The CFU/ml on antibiotic plates was compared to the CFU/ml without antibiotics. Samples with >90 CFU/ml were excluded.

Results: 23 sample pairs (raw and treated sewage) from 20 treatment plants were included. Treatment removed on average 98% of enterobacteria, but of those left, a larger percentage were resistant, for all antibiotics but ciprofloxacin (Table). One CTX-M-carrying E. coli was found; the oldest detected in Finland so far (in influx wastewater from Turku City, December 2001).

Conclusion: Sewage treatment, although removing most of the bacteria, can increase the frequency of resistant bacteria. Studying wastewater can also work as an early warning system, enabling the detection of emerging resistance in faecal bacteria.

<table>
<thead>
<tr>
<th>Percent resistant</th>
<th>Trimetoprim</th>
<th>Tetracycline</th>
<th>Ciprofloxacin</th>
<th>Nalidixic acid</th>
<th>Streptomycin</th>
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<tbody>
<tr>
<td>Influent</td>
<td>7.4</td>
<td>7.1</td>
<td>0.6</td>
<td>5.4</td>
<td>5.5</td>
</tr>
<tr>
<td>Effluent</td>
<td>11.5</td>
<td>15.2</td>
<td>0.7</td>
<td>7.5</td>
<td>8.8</td>
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</table>

Method: Lincolnshire is England's second largest county with an area of >500 hectares and a largely rural population of >1 million. It is covered by a single-managed microbiology service which uses UK Health Protection Agency national standard operating procedures, disc diffusion sensitivity testing to BSAC standards and collects sensitivity data on one IT database. This was interrogated for all urinary coliforms from hospital and community patients that required second line sensitivity testing over the period of March to August 2008 and sensitivities of these isolates to 16 routinely tested antibiotics were recorded.

Results: Of 16,944 urinary coliform isolates from hospitals and community tested March-August 2008, 806 (4.8%) were multi-resistant requiring second line sensitivity tests. Percentage sensitivities against 16 antibiotics are shown in the figure (AMC = amoxicillin clavulinate; AMX = amoxicillin; ATB = aztreonam; CAZ = ceftazidime; CFX = cefotaxime; CIP = ciprofloxacin; CPO = cefpodoxime; CTX = cefotaxime; CXM = cefuroxime; ERT = ertapenem; GEN = gentamicin; MEC = mecinillin; NIT = nitrofurantoin; TAZ = piperacillin-tazobactam; TEM = temocillin; TMP = trimethoprim). Sensitivity to temocillin, ertapenem and mecillinam was 95.8%, 95.4% and 83.6% for all multi-resistant isolates, 92.7%, 90.6% and 83.3% for all gentamicin resistant isolates (n = 96), 93.5%, 95.7% and 92.4% for all ciprofloxacin resistant isolates (n = 184). 108 (0.6%) of total isolates, 13.4% of resistant isolates were extended spectrum beta lactamase (ESBL) producers and 32 (0.2% of total, 4.0% of resistant) were Enterobacter, Citrobacter, Morganella, or Serratia genera. Sensitivities against these four genera and ESBLs were highest (>95%) for temocillin, ertapenem and mecillinam.

Number resistant coliforms tested

Conclusion: In our large, predominantly rural, population, temocillin and ertapenem show the lowest resistance rates of 16 antibiotics routinely tested in resistant urinary coliforms. For ESBL producers and Enterobacter, Citrobacter, Morganella, Serratia genera, temocillin, ertapenem and mecillinam have very low resistance rates. Mecillinam is available as an oral preparation but sensitivity testing in ESBLs may be unreliable. Temocillin and ertapenem are good options for the treatment of antibiotic multi-resistant urinary pathogens in hospital patients.

Surveillance of antibiotic resistance in urinary coliforms in Greater Lincolnshire, U.K. and the potential for temocillin, ertapenem and mecillinam usage


Objectives: Surveillance of antibiotic sensitivity patterns in resistant urinary coliforms for a large, rural population over a recent six month period.

Method: Lincolnshire is England's second largest county with an area of >500 hectares and a largely rural population of >1 million. It is covered by a single-managed microbiology service which uses UK Health Protection Agency national standard operating procedures, disc diffusion sensitivity testing to BSAC standards and collects sensitivity data on one IT database. This was interrogated for all urinary coliforms from hospital and community patients that required second line sensitivity testing over the period of March to August 2008 and sensitivities of these isolates to 16 routinely tested antibiotics were recorded.

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Surveillance of antibiotic resistance in urinary coliforms in Greater Lincolnshire, U.K. and the potential for temocillin, ertapenem and mecillinam usage


Objectives: Surveillance of antibiotic sensitivity patterns in resistant urinary coliforms for a large, rural population over a recent six month period.
Results: Using CLSI P. aeruginosa breakpoints (≤64 mg/L), P/T had the broadest coverage (% S) in two regions (EU, LA) and overall at 83.6% followed by MER (83.0%) > IMP (79.7%) > PIP (79.5%) > CPM (77.5%) > CAZ (75.8%). Other non-beta-lactam activity results (% S) were ciprofloxacin at only 71.5%, but tobramycin and polymyxin B had higher S rates (81.0 and 99.5%, respectively). Trends toward P/T resistance (R) were noted between 1997–1999 and 2000–2007 in APAC (−11.6%), NA (−4.0%) and EU (−2.3%). LA S rates were lowest but actually increased over time by +2.9%; current rate 79.4% S. For beta-lactamase inhibitor combinations S rates were higher for P/T when compared to PIP alone in all regions (+2.6 to 7.1%), greatest for APAC (+7.1%). R surveillance programs should be sustained to document emerging patterns of old and newer agents for difficult to treat pathogens such as P. aeruginosa.

Conclusions: P/T remained the most active beta-lactam tested in vitro against clinical isolates of P. aeruginosa found in the SENTRY Program (1997–2007). Trends toward slightly decreased S were noted in all regions over the decade, except LA; only polymyxins had S rates increased (1997–2007). Trends toward P/T resistance (R) were noted between 1997–1999 and 2000–2007 in APAC (−11.6%), NA (−4.0%) and EU (−2.3%). LA S rates were lowest but actually increased over time by +2.9%; current rate 79.4% S. For beta-lactamase inhibitor combinations S rates were higher for P/T when compared to PIP alone in all regions (+2.6 to 7.1%), greatest for APAC (+7.1%). R surveillance programs should be sustained to document emerging patterns of old and newer agents for difficult to treat pathogens such as P. aeruginosa.
were then analysed to assess the parameters being audited: the indication for CSU collection (clinical suspicion, costoverterbral tenderness or fever), change of catheter (+/- antibiotic prophylaxis) and antibiotic usage. Overall 41 patient records were collected over a 2 month period. 30/41 patients were female and the median age was 75 (range 38−98).

Results: Only 13/41 (32%) of samples originated from patients with possible symptomatic UTI. While 21/41 (52%) of catheters were removed, this occurred only in 6/13 (46%) of patients where symptomatic infection was suspected. Furthermore, 15 catheters were removed when not clinically indicated, and 6 of these were replaced, potentially introducing a second bacteraemic event. Of the 21 removed catheters, only 9 patients (43%) received gentamicin prophylaxis. With regards to treatment of suspected CAUTI, of the 20 patients that received antibiotics, only 8 (40%) had a clinical indication for doing so at the time of sample collection. Furthermore, there was no association between catheter removals and administration of antibiotics to treat a suspected UTI.

Conclusion: The variability in clinical practice identified by this audit have resulted in new hospital guidelines, specifying clear indications for CSU collection (fevers, localising tenderness, rigors) and gentamicin prophylaxis (history of CAUTI following catheter manipulation, recent catheterisation following urinary tract instrumentation, neutropenia).

An education programme has been rolled out to reinforce these new guidelines, prior to a comprehensive re-audit. This audit may serve as a template for other hospitals to compare local practice in the management of CAUTI to evidence based standards.

P684 Persistence of Escherichia coli clones in recurrent urinary tract infections
L. Skjot-Rasmussen*, A.M. Hammerum, C.H. Lester, L. Jakobsen, P. Larsen, N. Frimodt-Møller (Copenhagen, Koge, DK)

Objectives: Escherichia coli is the most frequent causative agent of recurrent urinary tract infections (rUTI). Relatively few studies have investigated the characteristics of E. coli strains causing rUTI, and differing views prevail as to whether rUTIs are primarily due to either reinfection with new strains or bacterial persistence or reinfection with the originally infecting strain. In the present study, E. coli isolates obtained from cases of rUTI were characterised with respect to antimicrobial resistance, phylogenetic group, virulence genes (VGs) and Pulsed Field Gel Electrophoresis (PFGE) typing.

Methods: From December 2005 through the beginning of April 2006, 131 E. coli urine isolates from Danish patients with UTI were collected at a general practice in Koge, Denmark. Of these, 41 isolates were isolated from the same 13 patients (2−7 isolates per patient). The 41 isolates from cases of rUTI were investigated by three multiplex PCR reactions for their phylogenetic background (A, B1, B2, D, non-typeable) and presence of eight VGs (kpsM2 II, iutA, papA, papC, hlyD, sfaS, focG, afa). PFGE-typing with XbaI restriction enzyme was performed, and MIC-values to eight antimicrobial agents (ciprofloxacin, nitrofurantoin, trimethoprim, sulfamethoxazole, ampicillin, chloramphenicol, tetracycline, and gentamicin) were determined.

Results: In ten out of the 13 patients with rUTI, index isolate and recurrences had similar PFGE patterns and belonged to the same phylogenetic groups, while in the remaining three patients they varied. Also, in these ten patients very high similarity of virulence gene profiles and antimicrobial resistance profiles among the individual isolates from the same patient was observed. In one patient, from whom four episodes of UTI was observed, the isolated strain changed from ciprofloxacin susceptible (isolate no. 1 and 2) to ciprofloxacin resistant (isolate no. 3) after treatment of the second UTI episode with ciprofloxacin. The isolate again turned ciprofloxacin susceptible for the fourth UTI episode (isolate no. 4).

Conclusion: Our study shows that episodes of rUTI are frequently attributable to bacterial persistence or reinfection with the originally infecting E. coli, i.e. one persisting clone. This may imply either an external reservoir (vaginal or rectal) for rUTI or the possible intracellular persistence of E. coli strains in the bladder epithelium.
An analysis of isolation of \textit{Mycoplasma hominis} and \textit{Ureaplasma urealyticum} from genital tract specimens of women receiving care at a general hospital serving rural population in Greece

A. Bakoxi*, E. Pitsouni, P. Holevas, E. Stamata, M. Falagias (Tripolis, Athens, GR)

Objective: \textit{Mycoplasma hominis} and \textit{Ureaplasma urealyticum} are among the most common microorganisms isolated from the genital tract of sexually active women. Furthermore, some experts perform screening for \textit{M. hominis} and \textit{U. urealyticum} in asymptomatic pregnant women with a history of preterm delivery.

Methods: We performed an analysis of microbiological data to evaluate the frequency of \textit{M. hominis} and \textit{U. urealyticum} isolation from genital fluid specimens of sexually active women, most of whom were pregnant, who received care at a general hospital of a small city in Greece serving mainly rural population. We retrospectively collected and analyzed the findings of the microbiological testing of cervical swab samples obtained from 796 young adult women (18–40 years old) receiving outpatient or inpatient care at the General Hospital of Tripolis, Tripolis, Greece (01/01/2001–01/01/2005) focusing on potential infection with \textit{M. hominis} and/or \textit{U. urealyticum}.

Results: The overall frequency of isolation of \textit{M. hominis} and \textit{U. urealyticum} in asymptomatic pregnant women (n=528) was 2% and 14%, respectively. The overall frequency of isolation of \textit{M. hominis} and \textit{U. urealyticum} in non-pregnant women (n=268) was 9% and 46%, respectively.

Discussion: Our study enriches the relevant literature, since it provides data regarding the frequency of isolation of these important genital pathogens for a tertiary centre in a small city in Greece serving rural population. The observed frequencies of \textit{M. hominis} and \textit{U. urealyticum} isolation are within the range of the figures reported in studies performed in various parts of the world.

Symptomatic women with non-gonococcal, non-chlamydial cervicitis show a high incidence of \textit{Mycoplasma genitalium} in Greece

I. Karansikolas, S. Baka*, S. Demeridou, G. Kaparos, P. Salomidou, V. Genimata, G. Arsenis, E. Koukouni (Athens, GR)

Objectives: The purpose of the present prospective study was to assess the incidence of \textit{Mycoplasma genitalium} (\textit{M. genitalium}) in a cohort of reproductive age women presenting with signs and symptoms of cervicitis since, to our knowledge, has not been previously reported in a Greek population.

Methods: Between March 2007 and March 2008 women of reproductive age presenting with signs and symptoms of cervicitis were invited to participate in the study. Eligible for our study were 150 women with non-gonococcal, non-chlamydial cervicitis which previously presented at different hospitals and private practices with the same symptoms and did not receive therapy for mycoplasmas. In order to identify aerobic microorganisms cervical specimens collected from all study participants were inoculated on blood agar, MacConkey agar, Chapman and Sabouraud agar followed by incubation at 37°C for 24 hours, whereas anaerobic cultures were carried out on Wilkins-Chalgren agar at 37°C for 48 hours. The isolated strains were identified using the automated system VITEK 2 (BioMerieux, France). For the identification of \textit{Ureaplasma urealyticum} and \textit{Mycoplasma hominis} we used Mycoplasm IST 2 (BioMerieux, France). Samples were tested for \textit{M. genitalium} using the hyplex® STD ID (BAG Health Care GmbH, Lich, Germany), a multiplex – PCR-ELISA system. Statistical analysis was performed using student t-test and chi-square test.

Results: \textit{M. genitalium} was detected in 27 (18%) of the 150 women tested. Data regarding women tested positive for \textit{M. genitalium} was compared to women who tested negative. The two groups did not differ in age (p=0.85), in the number of children (p=0.09) and in number of sexual partners (p=0.64). Interestingly, a higher proportion of women not using condoms had \textit{M. genitalium} isolated from their cervixes (p=0.021). Pruritus, in contrast to other symptoms, was more frequently associated with \textit{M. genitalium} cervicitis (p=0.05) while dyspareunia was significantly increased in the control group (p=0.05) where other pathogens except \textit{M. genitalium} were isolated. In 9 patients \textit{M. genitalium} was the only pathogen isolated while in 12 cases was isolated together with \textit{Ureaplasma urealyticum}. Finally, 6 patients presented with infection by all 3 mycoplasmas tested.

Conclusions: Symptomatic women harbour \textit{M. genitalium} in their lower genital tract. Rapid detection by means of a multiplex – PCR-ELISA system is useful for a prompt and correct management of these women.

Anorectal Chlamydia trachomatis infections in Swiss HIV-infected homosexual men


Objectives: Since 2003, anorectal lymphogranuloma venereum (LVG) and non-LGV \textit{Chlamydia trachomatis} infections are re-emerging among homosexual men in developed countries. We prospectively assessed the prevalence and risk factors for these infections in HIV-infected homosexual men in a large cohort, the Swiss HIV Cohort Study (SHCS).

Symptomatic women with non-gonococcal, non-chlamydial cervicitis show a high incidence of \textit{Mycoplasma genitalium} in Greece

I. Karansikolas, S. Baka*, S. Demeridou, G. Kaparos, P. Salomidou, V. Genimata, G. Arsenis, E. Koukouni (Athens, GR)
**Methods:** Male homosexual SHCS participants who reported unprotected receptive anal sex and/or symptoms of proctitis during a visit at one of the SHCS centres from April 2007 to March 2008 were eligible. Those enrolled consented to complete a questionnaire and to have an anal swab screened for *C. trachomatis* DNA by real-time TaqMan PCR. Positive samples were genotyped by ompA gene amplification and sequencing. Demographic, immunologic and virological data were retrieved from the SHCS database.

**Results:** 149 men were enrolled. 2 were excluded who did not fulfill inclusion criteria, leaving a total of 147 anal swabs from 147 men. The prevalence of anorectal *C. trachomatis* infection was 10.9% (95% confidence interval [CI] 6.2%-17.6%). Of the 16 *C. trachomatis*-positive swabs, one LGV was identified from a man presenting with a 7 day history of rectal discharge, tenesmus, and bloody stools. The remaining serotypes were G (n=5), J (4), E (2) and D (1). Serotype could not be determined in 3 samples. 5/16 men with versus 54/131 men without anorectal *C. trachomatis*-infection had detectable HIV viremia >40 copies/ml. In both anorectal *Chlamydia*-positive and negative groups, 19% of men reported symptoms of proctitis. Having had more than 20 sex partners within the last 2 years was the only identified risk factor for anorectal *C. trachomatis* infection (odds ratio 5.6, 95% CI 1.87–17.09). Neither infrequent use of condom with occasional partners, nor other risk factors for sexually transmitted infections (STIs), such as drug use, alcohol misuse, fisting, anal toy use or rimming, were associated with anorectal chlamydial infection.

**Conclusion:** In this HIV-infected population at high risk for STIs, the prevalence of anorectal chlamydial infection is moderate compared to other STIs and we found no evidence of an ongoing LGV outbreak. Nevertheless, since chlamydial infections are commonly asymptomatic and since the risk of transmission of other STIs is high, screening for anorectal *C. trachomatis* infection should be added to the other routine screening of homosexual men who report unprotected receptive anal sex.

**Objectives:** Emerging STIs are a continuing challenge for diagnosis. Known diseases emerge with new clinical presentations, like LGV in MSM, while diagnosis of others, like *M. genitalium* (Mg), becomes possible through new PCR technology.

**Methods:** Standard STI care was provided for all patients attending the STI-outpatient clinic. From January until June 2008, 1362 consecutive specimens submitted for STI-testing were collected ofresistance of*Neisseria gonorrhoeae*and*Chlamydia trachomatis*testing were collected and used for Mg DNA detection by real-time PCR. DNA was isolated from all specimens using NucliSENS reagents and easyMAG equipment. Primers and probe were previously validated (Jensen et al., 2004). When the Mg Ct value was >35, specimens were completely retested. Addition of phocine herpes virus (phHV) to the clinical specimens was used as an internal control for lysis and amplification. A phHV Ct value of >35.7 (historical mean plus 1.5×SD) was regarded as inhibition.

**Results:** One hundred fifteen specimens (8.4%) had a phHV Ct value >35.7 and Mg Ct value of >50 and were regarded as not suitable for PCR diagnosis. Of these specimens, 85 were self-collected vaginal swabs, 15 were cervical/urethral swabs, and four were urines. Of the remaining specimens, six had a Mg Ct value of >35, but were negative upon retesting. Thirty-two specimens had a Mg Ct value of >35 and were again positive on retesting. Thirty-five specimens had a Mg Ct value between 25 and 35. These 67 specimens (4.9%) were regarded as positive: one throat specimen, five penile swabs, seven cervical/urethral swabs, 10 rectal swabs, 21 urines, and 23 vaginal swabs. From 42 Mg positive patients (21 males and 21 females; age 18 to 62 years) further data were available. Twenty-five (60%) had no concurrent STI and 21 (50%) had no history of other STIs. Twenty-nine (69%) reported no symptoms and 10 (24%) one or more symptoms, two of which had another STI compatible with those symptoms. Thirty-three (79%) reported vaginal sex only, seven (17%) anal sex only, and one both types. In all but one case the sex was unprotected.

**Conclusion:** Mg is an emerging pathogen, that is claiming its place. In this study we have shown that the incidence of infections is at least 4%. Only one in every three patients will report symptoms. All infections are the outcome of unprotected sex. Thus, in empiric syndromic therapy the physician should take the possibility of an infection with Mg into account, especially when standard therapy for *Chlamydia trachomatis* fails to cure the symptoms.

**Micro-organisms isolated from male urethral exudates during an 8-year period in a Spanish teaching hospital**

M.C. Martínez*, D. Domingo, S. Agudo, M.J. Moreno, M. Lopez-Brea

**Aim:** The main aim of this study was to describe the microorganisms isolated from male urethral exudates, from 2000 to 2008 at the Microbiology Department of La Princesa University Hospital in Madrid.

**Methods:** Eight hundred and eighty six samples obtained from urethral exudates from men with symptoms of urethritis were collected by standard procedures. They were examined by Gram stain and inoculated on blood agar medium, chocolate agar medium and modified Thayer-Martin medium and incubated at 37°C in CO2 and 10% CO2 until 48 hours. Subsequently, they were identified by API NH (Biomerieux), by MicroScan (Dade Behring), CHROMagar Candida (Becton Dickinson/BBL) and Auxacolor.

**Results:** A total of 290 samples (32.73%) were considered of microbiological value and informed to the clinician. According to the positive samples, the percentage of isolated microorganisms were as follows: 14.45% of *Neisseria gonorrhoeae*, 4.17% of *Haemophilus parainfluenzae* (groups II and III), 5.64% of Gram-positive cocci, 4.74% of Gram-negative rods and 3.72% of Candida sp.

The average age for each group of microorganism isolated was: 33.78 for *N. gonorrhoeae*, 32.65 for *H. parainfluenzae*, 49.18 for Gram-positive cocci, 67.88 for Gram-negative rods and 66.10 for Candida sp. The resistance rate of *N. gonorrhoeae* to the antimicrobial agents studied, increased considerably during the period.

**Conclusions:** According to positive cultures, the most prevalent microorganism isolated from urethral exudates was *Neisseria gonorrhoeae*, followed by Gram-positive cocci, *Gram-negative rods, Haemophilus parainfluenzae* and finally Candida sp. By age group, *Neisseria gonorrhoeae* and *H. parainfluenzae* were more prevalent in young adults, Gram-positive cocci had a scattered distribution and Gram-negative rods and Candida sp. were isolated more frequently in elderly. The high rates of resistance of *N. gonorrhoeae* may difficult the treatment of gonococcal infection in the next years.

**Seroepidemiological survey of viral hepatitis, HIV, syphilis and herpes simplex virus in a Portuguese correctional facility**


**Objectives and Methods:** Prison inmates are reported to have higher rates of transmissible infectious diseases, particularly blood-borne virus and sexually transmitted infections than the general population. This cross-sectional study conducted during December 2007 to January 2008 and March to June 2008 was aimed to determine the seroprevalence for viral hepatitis (HAV, HBV, HCV), human immunodeficiency virus (HIV), syphilis and herpes simplex virus (HSV-1 and HSV-2) in prison inmates of a regional Portuguese prison. The following serological techniques were used: CMIA (“cheluminescent microparticle immun assay”) for viral hepatitis and HIV (confirmed by “western blot”); RPR (“rapid plasma reagin”) and TPPA (“Treponema pallidum particle agglutination assay”) for syphilis and ELISA (“enzyme-linked immunosorbent assay”) for HSV.
Results: During the study periods, 151 (71.6%) of 211 male inmates accepted to be screened for the mentioned infectious diseases. The mean age was 34.1 ± 10.8 [19−75] years. Anti-HAV was positive in 69.5% (n = 105). The rate of anti-HCV + was 34.4% (n = 52). One (0.7%) person had HBs Ag and 29 (19.2%) had past HBV infection (anti-HBc + anti-HBs). Nonimmune inmates for HBV were 40.4% (n = 61). Syphilis was diagnosed in 6.0% (n = 9). The rate of HIV infection was 6.6% (n = 10; all HIV-1). The majority (n = 8) of HIV-infected inmates were co-infected with HCV. The seropositivity of HSV-2 (most common cause of genital ulceration) was 19.9% (n = 30) and of HSV-1 was 82.1% (n = 124). Alcohol dependence was reported by 26.5% (n = 40). Excluding tobacco and prescription medication, 73.5% (n = 111) reported drug use in prison. The most commonly used drugs were: cannabis (100%; n = 111) followed by heroin (56.7%; n = 63). Methadone maintenance treatment was reported by 12.6% (n = 19). In prison, 43% (n = 65) had received a tattoo and 3.3% (n = 5) a piercing.

Conclusions: The rate of HCV antibody was noteworthy. Vaccination for HBV should be offered for nonimmunes. HIV infection rate (6.6%) in Coimbra’s Regional Prison is at least 13 to 22 times greater than in general population. As the inmate’s return to community increases the risk of disease exposure for the general population, early detection and counselling is urgently needed for prisoners.

This study was sponsored by a grant of Portuguese Society of Clinical Microbiology and Infectious Diseases – Abbott Virology.


A. Karantani*, A. Ioakimidou, A. Michailidou, S. Georgiouss, A. Chatzimichailidou (Giannitsa, GR)

Objectives: Syphilis is a sexually transmitted disease caused by *Treponema pallidum*. Transfusion syphilis was once a serious problem but nowadays cases are very rare in the Western World due to the parallel decline of the prevalence of the disease. In Greece, blood donors are being routinely screened for syphilis by non-treponemal serological tests such as rapid plasma reagin (RPR). The aim of this retrospective study was to determine the prevalence of syphilis among the healthy blood donors of the City of Giannitsa of Northern Greece during a ten-year period of time.

Methods: During the last decade (October 1998 to October 2008) sera from 25551 consecutive blood donors were screened for syphilis using a commercial non-treponemal serological test, the rapid plasma reagin-RPR (Omega Diagnostics, Scotland, United Kingdom). The RPR-positive samples were further examined by specific treponemal tests in a reference laboratory. The specific treponemal tests that were used were the *Treponema pallidum* haemagglutination test (TPHA) and the fluorescent treponemal antibody absorption test (FTA-ABS).

Results: During the study only one serum sample was found RPR-positive. The diagnosis of syphilis was confirmed by both TPHA and FTA-ABS tests. The blood donor was male without clinical symptoms and the diagnosis of “syphilis incognito” was made.

Conclusions: Although the practically zero prevalence of syphilis among the healthy blood donors of our area indicates that syphilis is not a major problem for transfusion medicine, the case of syphilis described above should keep alert the Blood Services of our area, especially when fresh blood components are needed.

**P693** Rising rates of syphilis in Bilbao health area between 2001 and 2007

P. Liendo, G. Espeleta, M. Perez, E. Esteban, R. Cisterna* (Bilbao, ES)

Introduction: The epidemiology of sexually transmitted infections (STI) is clearly related to many socio-cultural factors and major changes in STD epidemiology have been noted since the onset of the HAART therapy. A dramatic increase in frequency of different STIs was noted in Bilbao over the past six years.

Aim: The aim of this study is to describe the syphilis cases identified at the Serology Laboratory at Basurto Hospital (SLBH) between 2001 and 2007 in Bilbao health area.

Material and Methods: All serological specimens for syphilis testing between 2001 to 2007 from different medical providers located in Bilbao health area were included in this study. We used an ELISA IgG assay for syphilis serological screening and in positive cases a RPR and FTA syphilis confirmatory test was performed. All early syphilis cases (primary, secondary, and early latent syphilis) are interviewed by STI specialists regarding history of symptoms, demographics, risk behaviours, and partner contact information for case finding and partner notification. We used standard CDC contact periods as the time period of interest when interviewing patients.

We used Fisher’s Exact test to compare categorical variables and Wilcoxon rank sum test to examine differences in the number of sexual partners reported by patients. We also calculated rates of early and latent syphilis cases and trends in numbers of persons tested and diagnosed through screening and partner notification from 2001 to 2007.

Results: Early syphilis cases increased continuously from 2001 to 2007 with 87% occurring among men who have sex with men (MSM). Ninety five percent of cases were men and the average age were bigger than the age recorded in women cases. Four percent of patients were diagnosed of HIV infection in the same STI episode. Despite public awareness campaigns, increased publicly financed syphilis screening among MSM and intensified partner notification efforts, the prevalence of early syphilis cases among screened populations was low (13%) and most (67.9%) of syphilis cases were diagnosed after seeking care for symptoms.

The proportion of cases diagnosed through screening and partner notification did not significantly change during the evaluation period, but early syphilis incidence among MSM more than doubled between 2004 and 2007.

Conclusions: New, innovative approaches to syphilis control are needed.

**P694** A multi-centre prospective study of risk factors for Jarisch-Herxheimer reactions after penicillin therapy among persons with syphilis


Objectives: Risk factors for development of Jarisch-Herxheimer (JH) reactions are rarely investigated in persons with syphilis who received penicillin therapy according to treatment guidelines for syphilis. This study aimed to investigate the factors associated with JH reactions among persons with syphilis after receipt of standard penicillin therapy.

Methods: Between January 1, 2007 and December 31, 2008, persons diagnosed as having syphilis were enrolled in this observational study. Diagnosis of syphilis was made by elevation of Venereal Disease Research Laboratory (VDRL) titers followed by confirmation with *Treponema pallidum* haemagglutination antibody (TPHA) assays. Those blood specimens tested positive for anti-HIV antibody would be confirmed by Western-Blot. Penicillin was given for those persons with syphilis by following the treatment guidelines. The patients receiving penicillin were contacted by cell phone to inquire reactions following receipt of standard penicillin treatment. The JH reactions were defined as fever and/or exacerbation of skin maculopapular rash within 24 hours of receipt of penicillin therapy.

Results: During the 2-year study period, 125 HIV-infected persons and 61 HIV-uninfected persons who received penicillin for syphilis were enrolled. JH reactions developed in 28% (35/125) of the former group and 13.1% (8/61) of the latter group. In univariate analysis, we found that persons with non-latent (primary or secondary) stage of syphilis, higher VDRL titers (\(1:32\)), and having HIV infection were more likely to develop JH reactions than those with latent syphilis, lower VDRL titers and without HIV infection, respectively (all comparisons, \(p < 0.05\)). In multivariate logistic regression analysis, we found that non-latent stage was the only independent factor that was associated with the development of JH reactions, with an odds ratio of 5.72 (95% confidence
Evaluation of Chlamydia trachomatis and Neisseria gonorrhoeae infections in patients visiting gynecology department of hospitals in Delhi using an in-house PCR assay and ELISA-based method of detection

A. Patel*, D. Sachdev, P. Sachdeva, D. Saluja (New Delhi, IN)

Infection by Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) is asymptomatic especially in women. Although completely curable by antibiotic, undetected infections by CT and NG can lead to various complications and transmission of infection. In developing countries, STI laboratories are limited or absent. An early, affordable and rapid diagnosis will be a valuable tool for control of NG and CT. We have developed and evaluated an in house PCR and ELISA assays for CT and NG detection and show high prevalence of infections in females.

Objectives:
1. To develop sensitive, rapid and point of care test for diagnosis of CT and NG and its evaluation.
2. To study the prevalence of infection in females from low resource income.

Methodology:
1. For in house PCR assay unique gene sequences of CT and NG, were amplified from gDNA isolated from endocervical swabs of about 200 patients aging 15–42. Molecular beacons were used to increase the sensitivity of PCR.
2. Proteins unique to CT and NG were cloned and purified. Sera of patients was tested for antibodies against these proteins in ELISA format.
3. Sensitivity and specificity were calculated using commercial kit as gold standard.

Results:
Out of 200 patients 41 patients were positive for CT (21%) and 53 were positive for NG (27%) and 38 were co-infected (19%). Our results show high prevalence of infection of NG and CT and their evaluation.

Conclusion: Our results show high prevalence of infection of NG and CT in young females from low resource setting. The in house developed PCR and ELISA are highly sensitive and cost effective method for the diagnosis of NG and CT and can provide an alternative diagnostic method for management of genital infection in India and other developing countries.

Azithromycin-resistant Neisseria gonorrhoeae strains recently isolated in Italy

S. Starnino, P. Stefanello*, on behalf of the Italian Neisseria gonorrhoeae Study Group

Objectives: Azithromycin (AZM) is not routinely used to treat gonorrhoea in our country but it is widely employed to treat Chlamydia co-infection, as recommended by the CDC guidelines for treatment of sexually transmitted diseases. In this study, AZM susceptibility of 219 gonococcal strains, during the evaluation of the prevalence of antibiotic resistance in Italy from January 2007 through June 2008 and the associated patient demographics and clinical characteristics, were assessed.

Methods: Minimum inhibitory concentrations (MICs) of azithromycin (AZM), ciprofloxacin (CIP), ceftriaxone (CRO), penicillin (PEN), and tetracycline (TET) were determined by E-test method. The AZM resistant strains (MIC ≥ 1 mg/L) were genetically analyzed by Neisseria gonorrhoeae Multi Antigen Sequence Typing (NG-MAST) and Pulsed Field Gel Electrophoresis (PFGE).

Results: A total of 22 AZM gonococci resistant strains were found among 219 collected in the study period. Five of the 22 strains showed a high level of AZM resistance with MIC values of 128 or 256 mg/L. Two of the latter showed a multidrug resistance phenotype. In particular, one strain was resistant to CIP (8 mg/L), PEN (32 mg/L) and TET (128 mg/L) and the other to CIP (12 mg/L) and TET (256 mg/L). All the strains were fully susceptible to ceftriaxone. Moreover, the resistant strains were mainly (17 out of 22) isolated among men having sex with men (MSM), Italians and resident in Rome. Among all the resistant strains NG-MAST analysis revealed the presence of 14 different Sequence Types (STs). PFGE showed the presence of 4 clusters among strains isolated from patients with similar epidemiological characteristics.

Conclusions: This is the first report describing azithromycin resistant N. gonorrhoeae strains in Italy. Interestingly, a high level of resistance was detected for 5 of the 22 AZM resistant gonococci to penicillin. Genetic relatedness was found among some resistant strains. This study represents a reference point for future surveillance in Italy and suggests the need to add azithromycin in the antibiotic susceptibility panel for gonococcus to monitor the drug's efficacy with particular regard to people at high risk for sexually transmitted infections.
**Conclusion:** Mutations at multiple loci act in synergism with each other to confer high penicillin resistance to NG strains.

**Methods:** Primary screening of clinical specimen included nested PCR detection of two T. pallidum specific loci (tmpC, polA). In PCR positive samples, 23S rDNA was amplified. Nested PCR protocol to detect 23S rDNA gene was developed and direct Sanger sequencing of PCR products was applied to detect mutations in corresponding part of the 23S rDNA gene.

**Results:** The set of 28 clinical isolates with detectable genetic material were collected from 22 patients in the Czech Republic in the time period 2005–2008. 14 patients (63.6%) were infected with macrolide sensitive strains of T. pallidum. Four patients (18.2%) were diagnosed with a strain bearing the A2058G transition and four patients (18.2%) with a strain bearing the A2059G mutation (spiramycin treatment failure was reported in one of these patients). There was a complete concordance of multiple samples collected from the same patient.

**Conclusions:** Screening of clinical samples revealed a novel mutation at the position 2059 of the T. pallidum 23S rDNA gene. This mutation causes macrolide resistance in several other bacteria.

Our results show that macrolide resistant isolates of T. pallidum bearing A2058G and A2059G mutations are relatively abundant in the Czech Republic, possibly causing macrolide treatment failures in penicillin allergic patients.

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**Prevalence and antimicrobial susceptibilities of genital mycoplasmas in outpatients with clinical urogenital infections in Korea**

**Objectives:** The aim of present study was to evaluate the occurrence of Ureaplasma urealyticum and Mycoplasma hominis in patients with clinical urogenital infections and to determine the antimicrobial susceptibilities for the most suitable treatment strategy.

**Methods:** 963 vaginal or urethral swabs were collected from 535 outpatient women and 255 urethral swabs were collected from 239 men. The identification of the genital mycoplasmas as well as the antimicrobial testing were performed using A7 Mycoplasma agar plates (bioMerieux, Marcy l’Etoile, France) and the Mycoplasma IST 2 commercial Kit (bioMerieux, Marcy l’Etoile, France). A7 Mycoplasma agar plates were supplemented with 10% fetal bovine serum.

**Results:** Of the 294 positive specimens from women, 263 (89.5%) and 7 (2.4%) were positive for U. urealyticum and M. hominis as a single pathogen, respectively. Both urogenital mycoplasmas were grown in 5.4%. The majority of U. urealyticum isolated were susceptible to tetracycline, doxycycline and azithromycin (89.4%, 95.8% and 92.0%, respectively), while ciprofloxacin and ofloxacin proved to be inactive against most of the strains. All of the M. hominis isolates were completely susceptible to doxycycline and about half of the isolates were susceptible to ciprofloxacin, ofloxacin, erythromycin, azithromycin and clarithromycin. Of the mixed isolates of U. urealyticum and M. hominis, 68.8% were susceptible to doxycycline. 18 positive specimens were obtained from men and only 1 U. urealyticum strains were isolated, the susceptibilities of them resembled those of U. urealyticum from women. All of the strains of mycoplasma were 100% susceptible to pristinamycin. In every positive specimen the colonies of U. urealyticum or M. hominis were observed on A7 agar plate by direct microscopy.

**Conclusions:** Of the patients with suspected urogenital infections U. urealyticum or M. hominis were isolated in 33.5% of women and 7.5% of men. In the evaluation of antibiotic susceptibility, the higher resistance was obtained against ofloxacin and ciprofloxacin by U. urealyticum, and against erythromycin, azithromycin and clarithromycin by M. hominis. Mixed infection of U. urealyticum and M. hominis had higher resistance to most antibiotics. The present results suggested that doxycycline would be the first choice when empirical treatment is necessary in our hospital.

**Drug interaction studies**

**Evaluation of EDTA and dipicolinic acid, with and without the addition of zinc, in the detection of metallo-beta-lactamases**

**Objectives:** Carbapenem-resistant Gram-negative bacteria carrying transferable resistance genes are spreading worldwide, and to stop local epidemics we must be able to detect them. Phenotypic detection of carbapenemases belonging to the metallo-beta-lactamases (MBL) is problematic. We compared the performance of the enzyme inhibitors EDTA and DPA in different combinations with imipenem (IMI), meropenem (MRP) and ceftazidime (CAZ).

**Methods:** We tested 16 strains with known MBLs (11 VIM, 3 IMP, 1 GIM, 1 SPM), 27 with known serine beta-lactamases including TEM, SHV, CTX-M and GES, of which 16 were ESBLs, and 2 KPC carbapenemases, 24 IMI-resistant Pseudomonas aeruginosa strains with unknown resistance mechanisms, and 16 IMI-resistant Acinetobacter baumannii strains with different OXA-genes.

Confluent suspensions were plated onto Mueller-Hinton II agar with or without the addition of 70 μg/ml of ZnSO4 (1:20). Five Rosco tablets (CAZ, DPA, MRP, IMI+EDTA, IMI) were placed in a row, at a distance of 10 mm edge-to-edge to the next. A CAZ+DPA tablet was placed separately. IMI+EDTA zone diameters > 4 mm compared to IMI zones, and CAZ+DPA vs. CAZ were read as positive, as recommended by the manufacturer. Synergy (keyhole zones) between tablets (CAZ and DPA, DPA and MRP, MRP and EDTA, IMI and EDTA) was noted. Hydrolysis of IMI was measured spectrophotometrically for the P aeruginosa and A. baumannii strains. MBL genes were screened by PCR.

**Detection of metallo-beta-lactamases:** zone diameter differences between imipenem+EDTA, and ceftazidime vs. ceftazidime+DPA.

**Results:** IMI+EDTA zone mm differences detected all 16 known MBL strains, EDTA synergy only 9, CAZ-DPA zone mm differences detected 13, and DPA synergy 12. Zinc addition lowered the sensitivity of all methods, due to smaller zones. The serine beta-lactamases were generally interpreted as negative; DPA+zinic was worst with 4 false positive. The P aeruginosa strains (no MBL genes found) tested false positive in 10/24 cases both by EDTA and DPA; addition of zinc lowered the number of false positives (4/7). The P aeruginosa mm differences overlapped with those of the MBL strains (Figure). IMI was hydrolysed well by 1 strain and weakly by 5. The A. baumannii strains were false positive in 6/16 cases by EDTA; addition of zinc increased this to 11/16. With DPA there were no false positives; addition of zinc gave 1.
Evaluation of drug-drug interaction study of zabofloxacin in vivo

(Gyeongsan, Anyang, KR; San Diego, US)

Objectives: Zabofloxacin, a new fluoroquinolone (FQ) antibiotic in a phase II study for community-acquired pneumonia (CAP), has a broad spectrum and a great potential against Gram-positive bacteria including S. pneumoniae and some quinolone resistant bacteria. Although FQs are associated with a low incidence of CNS disorders, they may occasionally induced convulsive seizures, especially in patients receiving FQs in combination with non-steroidal anti-inflammatory drugs (NSAIDs), epileptic seizures in patients receiving both FQs and theophylline, and prolongation of prothrombin time (PT) due to a possible interaction between many FQs and warfarin. The purpose of this study was to investigate drug-drug interactions between zabofloxacin and NSAIDs, theophylline and warfarin.

Methods: Zabofloxacin and other FQs [gatifloxacin (gati), levofloxacin (levo), ciprofloxacin (cipro)] were administered once orally to ICR mice at doses of 1000 mg/kg (n = 6 or n = 7) with pretreatment of NSAIDs (fenbufen, BPAA, indomethacin, aspirin and celecoxib), theophylline and warfarin (once orally, 400 or 200 mg/kg). Mice receiving FQs with NSAIDs and theophylline were monitored for neurotoxic signs, such as tonic extensor, convulsion and mortality within 2 hours after the FQs dose. Mortality within a day was also monitored. In mice pretreated with warfarin, the PT was determined at 24 hrs after FQ dosing.

Results: Gati, cipro and cipro showed relatively severe neurotoxic signs when administered with NSAIDs and theophylline. In addition, these FQs demonstrated a significant prolongation of PT compared to that of vehicle control groups. No neurotoxic signs and prolongation of PT were observed in zabofloxacin-dose groups.

Conclusion: Zabofloxacin demonstrated a favourable drug-drug interaction profile compared to other FQs in ICR mouse when given to ICR mice pretreated with NSAIDs (fenbufen, BPAA, indomethacin, aspirin and celecoxib), theophylline and warfarin at doses of 200 and 400 mg/kg.

In vitro antibacterial and anti-pathogenic activity of colistin, azithromycin and their combinations against colistin-susceptible and -resistant Pseudomonas aeruginosa

C.H. Tan†, J Li, L. Turnbull, C.R. Whitchurch, J.D. Turnidge, R.L. Nation (Melbourne, AU)

Objectives: Infections caused by multidrug-resistant Pseudomonas aeruginosa (PA) have become a critical challenge and colistin (COL) is often used as ‘salvage’ therapy. The pathogenicity of PA is attributable to the arsenal of pathogenic factors, e.g. quorum sensing, biofilm and pyocyanin production. Azithromycin (AZM), has been shown to have beneficial effects on these factors but PA is resistant to AZM. The study evaluated COL and AZM, both alone and in combination, in regard to antibacterial activity and effects on pathogenic factors.

Methods: COL susceptible PA01 and 3 clinical isolates (COL°, MIC ≤ 2 mg/L) and 4 COL resistant isolates (COL°, MIC 8–128 mg/L) were studied; all had AZM MICs > 128 mg/L. Studies were performed in 96-well plates. Multiples of the COL MIC up to their respective MICs were used for COL°, 2.4, 8 mg/L COL were used (all clinically relevant). Clinically achievable 0.125, 0.25, 0.5, 1, 2 mg/L AZM were used. Untreated controls were included. Assays were conducted at 24h or 48h depending on the isolate. Antibacterial effect was measured by viable counting. Synergy was regarded as fractional inhibitory concentrations < 0.5. Analytical methods based on LC/MS and HPLC were developed to assay the quorum sensing molecule N-3-oxodecanoyl-homoserine lactone (C12-HSL) and pyocyanin, respectively (limits of quantification 0.5 mg/L and 0.2 mg/L, respectively). Biofilm was assessed using crystal violet.

Results: Synergy was bidirectional. A marked synergy was observed against COL°, e.g. decreasing the AZM MIC from >128 mg/L to 2 mg/L and COL MIC from 128 mg/L to 4 mg/L. Combinations had greater antibacterial activity, relative to COL and AZM alone, against COL°. For all isolates, sub-MIC AZM substantially reduced, in a concentration-dependent manner, the production of C12-HSL and pyocyanin (up to >90% reduction), an effect COL enhanced. COL at the MIC reduced by >90% the amount of biofilm formed by COL°, AZM effect was minimal. In general, COL° formed relatively poorly-stained biofilms; clinically relevant, but sub-MIC, concentrations of COL were without effect while 1 mg/L and/or 2 mg/L AZM usually increased biofilm formation. The effects of combinations on biofilm formation were variable across strains.

Conclusions: The findings showed the potential benefits of combining COL and AZM, particularly against COL°R. The effects on key pathogenic factors provide potentially important infection management strategies.

Interactions between linezolid and a carbapenem on methicillin-resistant Staphylococcus aureus


Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) is both a therapeutic and epidemiological challenge. Eradication from an infection site might require the use of potent antibiotics or the combination of different ones. In this study we analyse the performance of linezolid in combination with two carbapenems on two different clones of MRSA from our hospital, by means of a variation of the microdilution dynamic checkerboard (MDCh), that is, time-kill curves (TKC) in a microdilution fashion.

Methods: MICs for both MRSA clones were calculated by microdilution in cation-adjusted Muller-Hinton broth. MDch for meropenem and etrapenem were evaluated at concentrations ranging from 1/1024 to 1× MIC, combined with linezolid at 1 and 4× MIC in 200-microliter 96-well microtitre plates. Microtitre TKC were performed for 1×MIC of linezolid plus 1/32× MIC of each carbapenem (a different microtitre plate was seeded for each CFU/ml counting time). MRSA viable cells were counted at 0 and 24 hrs for the MDCh and at 0, 6 and 24 hrs for micro TKC, by seeding 100 microtitre dishes of each well on blood agar plates at 10-fold serial dilutions.

Results: Linezolid alone showed its maximum bacteriostatic effect at 4–8× MIC. MDCH showed a synergistic effect for the combination of 1× MIC of linezolid plus both carbapenems at concentrations ranging from 1/64 to 1/8× MIC. No synergy was observed in any of the combinations of linezolid at 4× MIC, moreover, a tendency towards antagonism was seen the higher the amount of carbapenem present in the combination. Discrepancies have been reported when different methods are employed, but micro TKC yielded similar results for the combinations carried out, without disagreement with the other two procedures, as for the MICs or the final effect.

Conclusions: Linezolid at 1× MIC in combination with a carbapenem under their MIC exhibited a synergistic effect on MRSA. This was not seen with higher concentrations of linezolid. Our work hypothesis for future studies is that linezolid at low concentrations allows some bacterial growth, but restrains mecA by means of inhibiting PBp2° formation, this lets carbapenems to act on the cell wall formation. Higher amounts of linezolid block bacterial metabolism, thus, no synergy can be seen.

On the other hand, micro TKC has proven to be material and time saving, and an easy and cheap procedure for basic research on drugs interaction, as well as teaching. It also offers the possibility for automation.

In vitro synergistic activity of ceftriaxone, rifampicin and doxycycline against Brucella melitensis isolates by E-Test


Objectives: Brucellosis is a zoonotic disease seen world-wide including Turkey. Ceftriaxone is used as a treatment option in neurobrucellosis
cases in our country. In our study in vitro synergistic activities of ceftriaxone-doxycycline and rifampicin were investigated.

**Methods:** In vitro activities of antimicrobial agent combinations were tested against *Brucella melitensis* strains isolated from 18 patients treated at Infectious Diseases and Clinical Microbiology Department of Ankara Research and Training Hospital. In vitro effectiveness of ceftriaxone-doxycycline, rifampicin- doxycycline and ceftriaxone-rifampicin combinations were tested by E test method. The fractional inhibitory concentration (FIC) index was calculated as FIC = /MICA-B/MICA+/MICB-A/MICB. The interactivity of in vitro combinations was evaluated as synergism, additive, indifference and antagonism depending on the conclusions of FIC index calculated for each strain.

**Results:** The MIC50 and MIC90 of isolates to ceftriaxone-doxycycline and rifampicin were 0.5 μg/ml and 0.75 μg/ml, 0.064 μg/ml and 0.125 μg/ml and 0.19 μg/ml and 0.38 μg/ml, respectively. Synergism was found in 14 strains (78%) with ceftriaxone-doxycycline combination and additive effect was detected in other 4 strain. Whereas only in one strain (6%) synergistic effect was seen between rifampicin and ceftriaxone combination. Additive and indifference effects were detected six and ten strains, respectively. Antagonism was found with this combination in one strain. With rifampicin-doxycycline combination, in 12 strains (63%) synergism, in five strains additive and in one strain indifference were found.

**Conclusion:** In our study, all Brucella isolates showed susceptibility to all the antibiotics tested. The results of this in vitro study suggest combination of ceftriaxone and doxycycline as a therapeutic alternative for neurobrucellosis.

**P705 In vitro activity of polymyxin B and rifampicin in combination against pandrug-resistant *Acinetobacter* spp.**

**T.P. Lim**, W. Lee, T.Y. Tan, S. Sasaki, T.T. Tran, L.Y. Hsu, A. Kwa

(Singapore, SG)

**Objectives:** Outbreaks of pandrug-resistant (PDR) *Acinetobacter* spp.(As) have emerged in Singapore. Combination therapy may be the only viable option until new antibiotics become available. While polymyxin B (PB) may remain a viable treatment option, heteroresistance has become a major problem. We evaluate if combination therapy with PB is warranted and the efficacy of PB and rifampicin (R) combined against PDR As isolated from our local hospitals, when combination therapy is needed.

**Methods:** 361 As isolates from all public hospitals in Singapore were collected from 2006–07 over 2 months each year and studied. MICs were determined according to CLSI guidelines and 29 PDR As isolates were identified. Emergence of resistance studies (ERS) were performed with approximately 10^7 CFU/ml at baseline against 3 isolates (selected based on the unique genotype that represents the PDR As population) with PB alone and in escalating concentrations. Serial samples were obtained over 5 days to determine total and resistant bacteria load. Resistant sub-population was detected using media plates supplemented with PB at 3 × MIC. (TKS) Time-kill studies (same baseline as ERS) were performed with approximately 10^7 CFU/ml at baseline against 3 isolates (selected based on the unique genotype that represents the PDR As population) with PB alone and in escalating concentrations. Serial samples were obtained over 5 days to determine total and resistant bacteria load. Resistant sub-population was detected using media plates supplemented with PB at 3 × MIC. (TKS) Time-kill studies (same baseline as ERS) were performed with the maximum, clinically achievable, unbound concentration (mcg/ml) of PB (2) and R (2) alone and in combination against the 29 PDR As isolates.

**Results:** All 29 PDR As isolates were susceptible to PB (MICs 1–2 mg/L) and resistant to all antibiotic classes whereas R MICs ranged from 2–16 mg/L. In ERS, a significant reduction in bacteria burden was seen for PB (1, 2, 4 mg/L). However regrowth was seen at 24 h due to selective amplification of a resistant sub-population(s) for all 3 PB regimens. Repeat MIC testing of the resistant isolates confirms PB resistance (MICs 32–128 mg/L). In TKS screening, PB was bactericidal after 2 h for all isolates; however, regrowth occurred within 24 hr. R was bacteriostatic with regrowth by 24 h in all isolates. PB+R achieved >99% kill from baseline in 16 out of 29 isolates with no regrowth at 24 h.

**Conclusions:** We have shown that our PB As has the propensity to exhibit heteroresistance, and combination therapy with PB is needed. These findings demonstrate that in vitro synergy of antibiotic combinations in PDR As may be strain dependant. PB and R may be a potential antibiotic combination as a pre-emptive therapy for PDR As infections and warrants further investigations.

**P706 Activity of vancomycin and daptomycin alone and in combination with gentamicin against *Enterococcus faecalis* interaction studies using a calorimetry assay**

**G. Munoz**, I. Majic, A. Steinhuber, A. Trampuz (Barcelona, ES; Basel, CH)

**Objectives:** Severe enterococcal infections and the antimicrobial resistance are increasing. Treatment options of these infections are limited. Vancomycin (VAN) and daptomycin (DAP) are active against enterococci, but the synergistic effect of gentamicin (GEN) is unclear. Calorimetry is a highly sensitive method for measurement of heat production generated by microbial growth. We determined the inhibition of heat production of *Enterococcus faecalis* ATCC 10433 incubated with VAN or DAP alone or in combination with GEN.

**Methods:** The MIC values of VAN, DAP and GEN were determined by a gradient strip test (E-Test). Subinhibitory concentrations between 0.062× and 0.5× the MIC were tested. For calorimetry, 4 ml glass ampoules were filled with 3 ml of TSB containing the respective antibiotic(s) and 0.1 ml saline containing 5 × 10^8 cfu/ml of the test strain. Heat generation of bacterial culture without antibiotic was used as control. Ampoules were air-tight sealed and heat production of the growing cultures at 37°C was measured continuously in a TAM III 48-channel batch microcalorimeter (TA Instruments, Newcastle, USA) over 6 h. The peak heat flow (in microWatt) with antibiotic(s) was recorded and compared with the peak without antibiotic(s). Experiments were performed in triplicate.

**Results:** The MIC values were 2 μg/ml for VAN, 1 μg/ml for DAP and 8 μg/ml for GEN. Calorimetry without antibiotics (control) showed a peak of 305±.15 microWatt (100%). When used alone (at 0.5× and 0.2× MIC), DAP inhibited growth-related heat production more efficiently than VAN (59% vs. 88% an 75% vs. 98%, respectively). The addition of GEN at low concentrations (0.125× and 0.062× MIC) showed an additional effect on growth-related heat production, whereas GEN alone at these concentration has no measurable effect on the heat flow curve (data not shown).

**Conclusions:** Using *E. faecalis* ATCC 10433 as test strain, the calorimetric assay showed that DAP alone was more active against VAN alone at the same subinhibitory concentration. The addition of GEN at low concentrations (≤0.125× MIC) augmented the anti-enterococcal activity, whereas GEN alone showed no antimicrobial effect at these concentrations. Calorimetry has the potential for a rapid and accurate evaluation of antimicrobial activity and their combinations.

<table>
<thead>
<tr>
<th>DAP (×MIC)</th>
<th>GEN (×MIC)</th>
<th>Peak heat flow</th>
<th>VAN (×MIC)</th>
<th>GEN (×MIC)</th>
<th>Peak heat flow</th>
</tr>
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<tr>
<td>0 0</td>
<td>100%</td>
<td>0 0 100%</td>
<td>0 0 100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 0</td>
<td>59%</td>
<td>0.5 0 88%</td>
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<td></td>
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<tr>
<td>0.5 0.125</td>
<td>54%</td>
<td>0.5 0.5 66%</td>
<td></td>
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<tr>
<td>0.5 0.062</td>
<td>58%</td>
<td>0.5 0.25 84%</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.25 0</td>
<td>75%</td>
<td>0.25 0 98%</td>
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<td></td>
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<tr>
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<td>72%</td>
<td>0.25 0.5 89%</td>
<td></td>
<td></td>
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<tr>
<td>0.25 0.062</td>
<td>74%</td>
<td>0.25 0.25 99%</td>
<td></td>
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</tr>
</tbody>
</table>

**P707 Evaluation of the in vitro activity of tigecycline alone and in combination with rifampicin against multidrug-resistant Gram-negative bacilli**

**F. Mzali**, V. Dubois, C. Vidalacce, A. Hourisangou, C. Quentin

(Bordeaux, FR)

**Objectives:** The in vitro activity of tigecycline (TIG) was evaluated against a collection of 107 Multi-drug resistant (MDR) Gram-negative
bacteria isolated from patients. TIG in vitro activity was compared to those of tetracycline (TET), doxycycline (DOX) and minocycline (MIN) and the antimicrobial activity of TIG combined with rifampicin (RIF) was assessed.

**Methods:** 107 genetically characterised non related MDR clinical strains were used in this study (77 Enterobacteriaceae, 18 Pseudomonas aeruginosa and 12 Acinetobacter baumannii). All isolates harboured various antibiotic resistant determinants singly or in combination (ESBIs, carbapenemases, chromosomal and plasmid mediated AmpCs). The MIC of TET, DOX, MIN, TIG and RIF were determined by an agar dilution method. The activity of TIG combined with RIF was tested by the checkerboard method. The effect of the drug combination (TIG + RIF) was estimated at the point of maximal effectiveness by the fractional inhibitory concentration (FIC) interpreted as follows: synergy (FIC < 0.5), addition (0.5 ≤ FIC ≤ 1), indifference (1 < FIC < 2) and antagonism (FIC ≥ 2). PCR using described primers was carried out to characterise the tetracycline resistance genes present in the strains.

**Results:** Regression curves between MIC values and zone diameters showed a good correlation: TET (r=0.967), DOX (r=0.930), TIG (r=0.949) and MIN (r=0.871). Based on MIC values, TIG showed a better in vitro activity than the other tetracyclines (MIC range of 0.015–16 mg/L). However, TIG as expected, proved to be less active against P. aeruginosa strains. The combination of TIG+RIF was in most cases additive, often synergistic and occasionally indifferent but never antagonistic. All strains were susceptible to TIG in the presence of concentration of RIF achievable in vivo except for the P. aeruginosa strains for which the combination was principally synergistic, but at concentrations of RIF of 4 mg/L and of TIG ≥2 mg/L which are higher than the recommended breakpoint. PCR showed the concomitant presence in the strains of various tetracycline resistance genes.

**Conclusions:** The results demonstrate the need to test for TIG on MDR clinical isolates. The excellent in vitro activity of TIG confirmed its clinical utility against these pathogens. Moreover, combination of TIG with RIF must be encouraged in order to increase its efficiency for the treatment of infections caused by MDR organisms and prevent the emergence of resistant mutants.

**P708** Mutant prevention concentration of tigecycline and vancomycin against contemporary clinical isolates of Clostridium difficile

J. Blondeau*, B. Barks, S. Borsos, S. Sanche (Saskatoon, CA)

**Objectives:** Clostridium difficile is an important cause of hospital acquired diarrhea (CDAD) and is the causative agent of pseudomembranous colitis. Numerous different classes of antibacterial agents have been associated with CDAD, however, in many instances the mechanism of the association remains unknown. We performed mutant prevention concentration (MPC) testing of CD isolates (collected September-December 2008) against tigecycline (TIG) and vancomycin (VAN).

**Methods:** Minimum inhibitory concentration (MIC) testing was based on current Clinical and Laboratory Standards Institute procedure by E-test using 105 cfu/ml on Brucella agar containing 5% sheep blood. Following incubation (anaerobic), the lowest drug concentration preventing growth was the MIC or MPC depending on the treatment of infections caused by MDR organisms and prevent the emergence of resistant mutants.

**Results:** For clinical isolates, MIC values (mg/L) for TIG ranged from 0.047 to 0.064 (4 strains). The MIC values (mg/L) for VAN were 0.25 to 0.38. 0.5. The MIC values for ATCC strain 6989 were 0.094 (TIG) and 0.5 (VAN). The MPC values (mg/L) were as follows: TIG – 0.063 for all strains; VAN – 2 to 4. MPC values for ATCC 6989 were 0.125 and 2 mg/L respectively. MIC and MPC values for TIG against ciprofloxacin resistant strain (MIC≥32 mg/L) were 0.047–0.094 and 0.063 mg/L respectively; 0.38–1.5 and 2 mg/L respectively for vancomycin.

**Conclusions:** TIG was highly active in vitro against contemporary CD isolates with MIC and MPC values ≤0.125 mg/L. VAN MPC values ranged from 2–4 mg/L. TIG showed a low propensity to selection for CD subpopulations with high MPC values. TIG may be useful for therapy in patients with CDAD.

**P709** Comparative minimum inhibitory concentration and mutant prevention concentration values of cethromycin, azithromycin, clarithromycin, erythromycin and telithromycin against clinical isolates of Streptococcus pneumoniae

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

**Objective:** Cethromycin (CET) is a ketolide antimicrobial agent with reported low minimum inhibitory concentration (MIC) values against macrolide susceptible and resistant pneumococcal strains, however, limited data exists on MPC activity. The mutant prevention concentration (MPC) defines the drug concentration necessary to block the growth of the least susceptible cell present in bacterial population ≥106 CFUs. We compared MIC and MPC values for CET against macrolide susceptible and resistant Streptococcus pneumoniae (SP).

**Methods:** For MIC testing, the recommended Clinical and Laboratory Standards Institute procedure was followed utilising 106 cfu/ml tested against double drug dilutions in Todd-Hewitt broth with inoculums at 35–37 degrees Celsius in 5% CO2 for 18–24 hours. For MPC testing, >106 CFUs were added to drug containing agar plates (tryptic soy agar with 5% sheep red blood cells). Inoculated plates were incubated as described for 24–48 hours and screened for growth. The lowest drug concentration preventing growth was the MIC or MPC depending on method.

**Results:** For 26 clinical isolates, MIC50/90 values for CET, azithromycin (AZ), clarithromycin (CL), erythromycin (ER), telithromycin (TEL) respectively were 0.004/0.008, 0.125/0.25, 0.031/0.063, 0.063/0.063, 0.008/0.016; MPC50/90 values respectively were 0.063/0.125, 1/8, 0.125/4, 0.25/1, 0.031/0.063. CET MPC values ranged from 0.002–0.125 for 10/11 strains with elevated MPC values to AZ. One strain with a MPC to AZ of ≥128 mg/L had a CET MPC of ≥2mg/L and TEL MPC of ≥0.063 mg/L.

**Conclusion:** CET had low MIC (MIC90 0.008 mg/L) and MPC (MPC90 0.125) values against macrolide susceptible and resistant SP. CET MPC90 value against AZ resistant isolates was 0.063 mg/L. CET and TEL had comparable MPC90 values (0.125 vs 0.063 mg/L). CET demonstrates a low propensity to select for ketolide resistant SP and may be useful for therapy against macrolide resistant strains.

**P710** Comparative study of modified microbroth dilution to agar dilution for determining the mutant prevention concentration of gatifloxacin and moxifloxacin against Streptococcus pneumoniae ATCC 49619

C. Hejje, J. Blondeau* (Saskatoon, CA)

**Objectives:** The current method (agar dilution) for mutant prevention concentration (MPC) testing of Streptococcus pneumoniae (SP) is technically more demanding than minimum inhibitory concentration (MIC) testing as subculturing to liquid media, subsequent incubation and then centrifugation are required to achieve organism densities necessary for the assay. This adds 2 days to obtaining a result. We compared a modified microbroth dilution method to agar dilution for determining MPC values for SP ATCC 49619 against gatifloxacin (Gfx) and moxifloxacin (Mfx).

**Methods:** For mutant prevention concentration (MPC) testing, >106 CFUs of SP ATCC 49619 were inoculated to drug containing agar plates (tryptic soy agar) containing gatifloxacin and moxifloxacin (10−106 CFU/ml) and incubated under ideal conditions (5% CO2 at 35–37 Degree Celsius) for 24–48 hours. For the modified microbroth dilution method to agar dilution for determining MPC values for SP ATCC 49619 against gatifloxacin (Gfx) and moxifloxacin (Mfx).

**Results:** MPC values for Gfx and Mfx by agar dilution was 0.5 mg/L. For Gfx, MIC (MPC) values for the 10−106 cfu/ml inocula was 0.125 mg/L, 0.25 mg/L for the 10−5–106 cfu/ml inocula and 0.5 mg/L for the
Toxoplasmosis and borreliosis: diagnostic and clinical problems

**P711** Evaluation of enzygnost IgA conjugate in combination with the kit Enzygnost Toxoplasmosis IgG (Siemens Healthcare Diagnostics) for the detection of IgA anti-Toxoplasma gondii in human serum samples

A. Marangoni*, A. Moroni, S. Accardo, R. Cevenini (Bologna, IT)

**Objectives:** Laboratory diagnosis of Toxoplasmosis is mainly based on serological methods, particularly important in the most challenging situations, as diagnosis of primary infection during pregnancy and diagnosis of congenital infection. Tests for the detection of IgA antibodies are especially important in the newborns, because they are more sensitive than IgM conventional methods. The purpose of this study was to evaluate diagnostic performances of Enzygnost system for IgA detection, achieved by using Enzygnost Anti-human IgA/POD Conjugate in combination with Enzygnost Toxoplasmosis IgG (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany).

**Methods:** A retrospective study was performed with 591 serum samples submitted to the Microbiology Laboratory of S. Orsola Hospital in Bologna for Toxoplasmosis screening. All the sera were tested by Enzygnost Toxoplasmosis IgG, Enzygnost Toxoplasmosis IgM and Enzygnost system for IgA (Siemens Healthcare Diagnostics). Border-Line or positive IgM results were confirmed by Vidas Toxo IgM (bioMérieux, Marcy l’Etoile, France). Finally, IgG avidity was performed by Vidas Toxo IgG Avidity (bioMérieux, Marcy l’Etoile, France) and LDBio Toxoplasma WB IgG/IgM (LDBio Diagnostics, Lyon, France).

Moreover, a second study was performed with 172 selected sera in order to compare results obtained by Enzygnost system for IgA with those obtained by Enzywell Toxoplasma IgA (Diesse, Monteriggioni, Siena, Italy).

**Results:** Retrospective study. 453/591 samples were negative, 53 were Border-Line and 85 were positive when tested by Enzygnost system for IgA. 3 babies were correctly diagnosed as infected infants because of the presence of IgA antibodies at birth. These newborns had negative IgM results in both conventional methods used, whereas comparative WB confirmed the infection because of the different immunological profiles between maternal and newborn samples. Comparative study. Results obtained by the two methods are summarised in Table 1. They showed a concordance of 93%.

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. of samples</th>
<th>No. of reactive samples when tested by Enzygnost and Enzywell, respectively (%)</th>
<th>% Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>97</td>
<td>41 (71.3) 8 (14.0) 5 (10.2) 3 (6.1)</td>
<td>80.0</td>
</tr>
<tr>
<td>Infants</td>
<td>32</td>
<td>2 (0.0) 32 (100) 0 (0.0) 0 (0.0)</td>
<td>100</td>
</tr>
<tr>
<td>HIV positive</td>
<td>26</td>
<td>5 (19.2) 21 (80.8) 0 (0.0) 1 (3.8)</td>
<td>96.2</td>
</tr>
<tr>
<td>Healthy blood donors</td>
<td>57</td>
<td>57 (100) 0 (0.0) 5 (1.9) 6 (1.9)</td>
<td>94.7</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>44 (25.8) 116 (67.4) 7 (4.1) 5 (2.3)</td>
<td>93.0</td>
</tr>
</tbody>
</table>

* = immunological situation of the patient:
- All the program woman had low avidity and IgM positive results.
- No infants born to mothers with primary infection during their pregnancies were infected by Congenital Toxoplasmosis (all of them turned to be seronegative within 1 year of age).
- All HIV positive patients had positive IgG results. The 3 patients with IgA positive results by both methods were also IgM positive.
- The healthy blood donors were selected to be IgG and IgM seronegative for Toxoplasmosis

Conclusion: Enzygnost system for IgA anti-Toxoplasma showed very good diagnostic performances: in the retrospective study it allowed the correct identification of three infected newborns and in the comparative study it showed a higher specificity than Enzywell Toxoplasma IgA, since no sera from healthy blood donors were scored reactive. We conclude that its good performances and its suitability for automation make it an ideal screening test.

**P712** Congenital toxoplasmosis in the Netherlands

L.M. Kortbeek*, Y van Duynhoven, C. Nijhuis, A. Havelaar, A. Hofnais (Bilthoven, NL)

**Introduction:** Congenital Toxoplasma (CT) infection may occur after primary Toxoplasma infection during pregnancy and can cause severe complications. The information available on the incidence of CT in the Netherlands are old data from a regional study in 1987 (Toxoplasma Intervention Prevention (TIP) study). In order to get more recent information on the incidence of CT in the Netherlands we conducted a study in neonates and used this to recalculate the burden of disease of CT in Daily Adjusted Life Years (DALY’s).

**Methods:** A random sample of dried blood spot filter paper cards from newborns born in 2006 in the Netherlands were tested for Toxoplasma gondii-specific IgM antibodies, using Wallac AutoDELFIA Neonatal Toxoplasma Screening kits and confirmed by a modified immunosorbent agglutination assay (ISAGA bioMérieux) for Toxoplasma specific IgM antibodies.

**Results:** Approximately 185,000 neonates were born in the Netherlands in 2006 and a random sample of 10,008 cards was tested. Thirty-two samples (0.32%) tested positive in the screening assay, eighteen samples were confirmed IgM positive, resulting in an observed birth incidence of 1.8 per 1000 live born children in the Netherlands in 2006. Accounting for 99.9% specificity and 77.7% sensitivity, the adjusted incidence would be 2.0 per 1000. This means that 388 infected children were born in 2006. Although regional differences were not statistically significant, the incidence of congenital toxoplasmosis appeared to be higher in the South-East and West than in the North-East of the Netherlands. The most likely burden of disease is 2227 DALY’s with a range of 818–6713 DALY’s. In the previous calculations using the incidence of the Dutch TIP study of 1987 this was 620 (range 220–1900) DALY’s.

**Conclusion:** The incidence of congenital toxoplasmosis in the Netherlands is much higher than previously reported with 2 infected children per 1000 live born. This is ten times higher than in Denmark (0.2 per 1000, Schmidt2006) and 20 times higher than in Ireland (1 per 10,000; Philip Mayne, personal communication) using the same methods. There is no screening program in the Netherlands and most children will be born asymptomatic and therefore will not be detected or treated.

After recalculation of the DALY’s of toxoplasmosis, the burden of disease of congenital toxoplasmosis in the Netherlands is high and if combined with acquired toxoplasmosis it will be even higher, indicating its significance as a zoonotic pathogen.

**P713** Binding properties of decorin-binding proteins from three different Borrelia genospecies

J. Newcomen*, M.K. Viljanen, J. Hytönen (Turku, FI)

**Objectives:** Lyme borreliosis (LB) is a tick born infectious disease which is caused by *Borrelia burgdorferi* sensu lato bacteria. There are three major Borrelia genospecies, *B. burgdorferi* sensu stricto (Bbss), *B. garinii* (Bg) and *B. afzelii* (Ba), which are known to cause disease in humans. *Borrelia* has several surface proteins which mediate attachment to different tissues and molecules in the tick or mammalian host. Decorin binding proteins (Dbps) A and B (DbpA and B) are two adhesins of *Borrelia* that are expressed during mammalian infection. They mediate bacterial attachment to proteoglycan decorin which is closely associated with collagen fibers in the extracellular matrix. Decorin is widely expressed throughout the body, and highest concentrations are detected in the skin and joints. Different *Borrelia* genospecies have different
Patients after proven neuroborreliosis – how severe is the persisting neuropsychological damage?


Objectives: Next to neurological sequelae such as persisting facial palsy or radicular symptoms, patients after Neuroborreliosis (NB) often report cognitive disturbances even if they had received an early and state-of-the-art antibiotic treatment. In this study, we evaluate for the first time in Europe the frequency and extent of these deficits in a sufficiently powered study.

Methods: 54 patients who had been treated during the last ten years in the Göttingen University hospital for proven Neuroborreliosis received an extensive standardised neurological examination, a three-hours neuropsychological work-up covering all important cognitive domains, quality of life questionnaires, questionnaires for psychological symptom-load, and a 3-Tesla MR-Scan with a 3D-T1 sequence to measure the brain volume. The MRI examinations were analyzed for atrophy using the FSL-SIENAX software package. All results were compared with an age-, education- and gender-adapted group of 33 neurologically healthy control persons.

Results: Patients after NB showed more often pathological findings in the neurological examination (mean±SD Scolpss Neurological rating scale 97.2±4.5 vs. 99.4±1.7, p < 0.01) but these neurological deficits were in general mild and only rarely disabling. Considering the cognitive functions, z-values were statistically lower in the NB group only in the domain of non-verbal learning/memory (mean±SD = –0.13±0.41 vs. 0.13±0.57, p = 0.02) and in the domain of frontal executive functions (mean±SD = –0.28±0.61 vs. 0.09±0.63; p < 0.01). However, in the examined domains, the difference of the frequencies of pathological results never reached significance. Quality of life scale values and the load of psychological symptoms as measured with the SCL 90-R questionnaires were also comparable with the control group. The total brain volume and the volumes of peripheral grey matter, total grey matter, total white matter and ventricular volume were not different when compared with the control group.

Conclusion: Statistically, Neuroborreliosis may lead to measurable neurological and to some extent also to neuropsychological sequelae. In most of the patients, however, these deficits are subtle and do not affect the quality of life on the long run. Alike, in our population, brain atrophy is rather the exception than the rule in patients after proven Neuroborreliosis.

Molecular analysis of Borrelia spirochetes detected in Ixodes granulatus ticks removed from rodents in Taiwan

C.M. Shih*, L.L. Chao (Taipei, TW)

Objectives: To identify the genetic identity of Borrelia spirochetes detected in Ixodes granulatus ticks removed from rodents in Taiwan.

Methods: A general survey was conducted to collect adult I. granulatus ticks removed from trapped rodents in Taiwan. Total genomic DNA was extracted from individual tick specimens by using DNeasy Tissue Kit (Qiagen). The genetic identity of Borrelia spirochetes were determined by analysing the gene sequences amplified by a genospecies-specific polymerase chain reaction (PCR) assay based on the 5S-23S intergenic spacer amplon gene of Borrelia spirochetes. Aligned sequences were analyzed by neighbour-joining (NJ) compared with maximum parsimony (MP) methods to estimate the phylogenetic relationships of these detected spirochetes.

Results: A total of 162 adult I. granulatus ticks were examined and tested by PCR assay and Borrelia spirochetes were detected in 71 adult ticks. Phylogenetic analysis reveals that all these detected spirochetes constitute two major separate clades from other Borrelia genospecies in both NJ and MP methods. Within the clades, 8 strains of Borrelia spirochetes detected in adult I. granulatus ticks were closely related to the genospecies of B. burgdorferi sensu stricto and 26 strains of detected spirochetes were closely related to B. valaisiana.

Conclusion: Our results describe the first detection of B. burgdorferi sensu stricto and B. valaisiana-related spirochetes in adult I. granulatus ticks collected in Taiwan. The genetic identity of these spirochetes was confirmed by analysing sequence homology of 5S-23S intergenic spacer gene. Further investigations on Borrelia spirochetes detected in patients, other ticks, and reservoir hosts would beneficial to the better understanding of genetic diversity of Borrelia spirochetes in Taiwan.

Performance of four commercial immunoblots for serological confirmation of Lyme borreliosis in Rhone-Alpes (France)

K. Barral*, C. Roare-Sobas, A. Carricajo, S. Tigaud (Lyon, Saint-Etienne, FR)

Objectives: Immunoblot (IB) is usually used to confirm antibodies specificity after a screening positive Lyme test. Many blots are commercially available but of our knowledge no evaluation has compared the results obtained by the different tests. This study aimed to analyse detection of immune response by four different serological confirmation tests.

Methods: The present study compared four IB IgG and IgM tests: Europe Line® (Virotech, Ingen), Ecolob® (Virotech, Meridian), EU Lyme WB® (MarDx, Trinity Biotech) and Recomblot® (Mikrogen, Diasonir). From May and December 2007, we tested the sera from 42 well-defined patients at different stages of Lyme borreliosis (clinical and serological findings), 10 patients with an indeterminate status and 33 non confirmed diagnosis. All sera were positive or equivocal on screening Enzygmost® (Dade-Berhing) test for borrelial IgG and/or IgM antibodies. Reactivity of VlsE (IgG) and OspC (IgM) were especially studied. Technical criterias were also evaluated such as automated use, simplicity of reading, and cost of use.

Results: Presence of borrelial IgG antibodies in patients sera was established in 68.2% (n=58), 76.5% (n=65), 60% (n=51) and 60% (n=51) for Europe Line®, Ecolob®, EU Lyme WB® and Recomblot® respectively. Borrelial IgM antibodies were found in 52.9% (n=45), 42.4% (n=36), 78.8% (n=67) and 55.3% (n=47) respectively. Among 42 confirmed Lyme borreliosis (LB) patients, concordant results for IgG were found in 59.5% (n=25), 64.3% (n=27), 54.8% (n=23) and 66.7% (n=28) with Europe Line®, Ecolob®, EU Lyme WB® and Recomblot® respectively. Likewise, we obtained for IgM 59.0% (n=25), 54.7% (n=23), 69% (n=29) and 71.4% (n=30) respectively. The diagnosis sensitivity evaluated for Europe Line®, Ecolob®, EU Lyme WB® and Recomblot® were 59.5%, 64.7%, 54.8%, 66.7% for IgG respectively and 59.5%, 54.8%, 69%, 71.4% for IgM antibodies respectively.

Conclusion: LB diagnosis remained difficult. IB results should be interpreted carefully always in relation to the clinical findings.
The polycyclic aromatic hydrocarbons modify response of lung epithelial cells to Aspergillus fumigatus spores, in vitro


Objective: Epidemiologic studies have shown association between elevated levels of polycyclic aromatic hydrocarbons (PAHs) in air and increased incidence of pulmonary infections, especially in patients susceptible to infection. We investigated influence of PAHs extracted from diesel exhaust on activation of signal transducer and activator of transcription STAT-1 and STAT-3 factors in lung epithelial cells infected with Aspergillus fumigatus spores, in vitro. The STAT-1 and 3 proteins are activated in response to microbial infections through respectively cytokine receptors and regulate production of inflammatory mediators. Interferons are activators of STAT-1, and interleukin-6 is a STAT-3 activator.

Methods: In our experiments human epithelial cells A549 were preincubated for 24 hours, with non-toxic concentration of PAHs, subsequently were treated with Aspergillus fumigatus spores for 24, 48 and 7 days. The activation of STATs were determined by an immunocytochemical method. The cells expressing STAT in the nucleus were labelled as STAT(+) cells. The activation of STAT system in A549 cells was expressed as the percentage of STAT(+) cells; the ratio of STAT(+) to STAT(-) cells.

Results: The PAHs solution contents 51% fenanthrene 30% acenaphthene 9% fluorene and acenaphene, fluoreanthene 5% weakly activated of STATs in lung epithelial A549 cells. The STAT-1 and 3 activation in A549 cells incubated with the Aspergillus fumigatus spores was significantly higher than in cells treated with PAHs only or control cells. In the cells stimulated simultaneously with PAHs and spores the activation of these transcription factors were higher than in cells incubated with fungal spores only, but not statistically significant.

Conclusion: These preliminary results demonstrate that PAHs may cause lung epithelial dysfunction and enhance inflammatory reaction to fungal antigens. The prolonged higher level of inflammatory mediators may contribute to the increased risk of degeneration pulmonary cells.

Echinacea purpurea herba inhibits adhesion and invasion of Streptococcus pyogenes adhering to and invading human mucosal epithelial cells

A. Conrad*, I. Engels, U. Frank (Freiburg, DE)

Objective: Streptococcus pyogenes is an important bacterial pathogen, causing infections of the respiratory and other organ systems in susceptible hosts. S. pyogenes infection is initiated by adhesion to and invasion of mucosal epithelial cells. An in-vitro model system for bacterial adhesion and invasion of respiratory epithelial cells was used to investigate the influence of a phytotherapeutic preparation containing the pressed juice of purple coneflower herb (Echinacea purpurea herba; Echinacin® Liquidum, Madaus GmbH, Cologne, Germany) as active agent on S. pyogenes epithelial cell adhesion and invasion.

Methods: Adhesion of S. pyogenes to human epithelial HEp-2 cells was determined by a flow cytometric assay. S. pyogenes (DSM 2071) was stained with Calcein-AM and adhesion kinetics were determined by incubating bacteria and epithelial cells for 30 min, 60 min, 120 min, and 180 min, respectively. For cell invasion, HEp-2 cell monolayers were infected with S. pyogenes (DSM 2071). After co-incubation for 30 min, 60 min, 120 min, and 180 min invasion of HEp-2 cells was analyzed by a penicillin/gentamicin-protection assay. The phyto-lyophilisate was applied at concentrations of 0, 10, 100, and 1,000 microg/mL.

Results: The administration of 1,000 microg/mL of the Echinaceae-preparation reduced S. pyogenes adhesion to epithelial cells on average by 18.3% at 0 min, 19.2% at 30 min, 22.6% at 60 min, 23.5% at 120 min, and 21.8% at 180 min (n=10, p<0.001, repeated measures ANOVA). HEp-2 cell invasion was decreased by 22.9% at 30 min, 38.6% at 60 min, 40.2% at 120 min, and 44.8% at 180 min respectively (n=11, p=0.008, repeated measures ANOVA).

Conclusion: The Echinacea purpurea herba preparation reduced significantly S. pyogenes adhesion and invasion of human epithelial cells. Our results hold significant potential for these preparations as therapeutic agents for the prophylaxis of streptococcal infection.

Potentially probiotic bacteria induce inflammasome activation and cytokine production in human monocyte-derived macrophages

S. Latvala*, M. Miettinen, R. Kekkonen, R. Korpela, I. Julkunen (Helsinki, FI)

Objectives: In the present study, we have analyzed the ability of eleven potentially probiotic bacteria to activate human monocyte-derived macrophages (MO) and induce their cytokine gene expression. Our aim was to analyze whether there are significant differences in the ability of these bacteria to activate macrophage inflammasome-induced IL-1β production, and whether suppressor of cytokine signaling (SOCS3)-mediated negative feedback systems are also operative.

Methods: MOs obtained from buffy coats and differentiated in vitro with GM-CSF were stimulated with probiotic bacteria. After bacterial stimulation the cell culture supernatants were collected and cytokine levels were determined by ELISA. The kinetics of mRNA expression of cytokine genes and the involvement of SOCS3 in MO responses were analyzed by qRT-PCR.

Results: Most bacteria induced cytokine production in a dose-dependent manner. However, certain differences in the ability of these bacteria to induce MO cytokine responses were found. All bacteria induced pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) indicating that macrophage inflammasome system was activated. In addition, some bacteria also induced anti-inflammatory (IL-10) and Th1 (IFN-γ) cytokines. Bifidobacterium, Streptococcus, and Lactobacillus strains were good inducers of IL-6, IL-10, and TNF-α, while Leuconostoc mesenteroides ssp. cremoris and Propionibacterium freudenreichii ssp. shermanii were relatively poor inducers of cytokine gene expression. In addition to activating cytokine production all studied bacteria were also able to induce SOCS3 gene expression, which likely leads negative feedback of extensive cytokine production in bacteria stimulated macrophages. SOCS3 gene expression was induced directly by bacterial stimulation as well as indirectly via IL-10 produced by macrophages.

Conclusion: Most probiotic bacteria activate macrophage inflammasome system leading to IL-1β secretion. In addition, other pro-inflammatory Th1 type cytokines and anti-inflammatory IL-10 were also efficiently induced in cells stimulated with these bacteria. All bacteria were also able to induce SOCS3 expression, which is likely to shut off enhanced cytokine gene expression. These results show that macrophages respond very strongly to bacterial stimulation even in the case of non-pathogenic bacteria and the responses vary between different bacterial strains.

Toll-like receptor ligand induced synergistic interferon gene expression in human monocyte-derived dendritic cells

S.M. Måkelä*, P. Osterlund, T.E. Pietilä, I. Julkunen (Helsinki, FI)

Objectives: Toll-like receptors (TLRs) are pattern-recognition receptors of the innate immune system that recognize various pathogen-associated molecules. TLR ligands are potent vaccine adjuvants and binding of ligands to different TLRs can induce a synergistic production of pro-inflammatory cytokines. In the present study, we have analyzed dendritic cell (DC) interferon (IFN) responses to the stimulation with two TLR ligands simultaneously.

Methods: Monocytes from healthy blood donors were differentiated into DCs in the presence of granulocyte-macrophage colony stimulating factors.
Detection of HIV-specific cytotoxic T-lymphocytes using viral delivery system

Y. Zeng, K.C.W Chan, C.S. Chan, F. Ng, K.H. Wong, B. Zheng* (Hong Kong, CN)

Objective: Although methods for the detection of HIV specific cytotoxic T lymphocytes (CTL) have been established, i.e. flow cytometry, Tetramer and Elispot using specific CTL peptides, their applications are still limited because: (I) the patients’ HLA-I subtypes must be determined; (II) only a few HLA-I HIV specific peptides have been identified; and (III) HLA typing and synthesis of specific peptides are expensive. Thus, this study aims to develop a new technique for detection of HIV specific CTL.

Methods: Recombinant adeno-associated virus (rAAV) expressing HIV antigens gp120, gp41 and gag were constructed. HIV antigen-specific CTLs were detected in 10 HIV patients with HLA-I subtype A2 (HLA-A2) by a standard method of flow cytometry for detection of intracellular IFN-γ using HLA-A2-restricted HIV-1 CTL specific peptides (gp120 194–202, gp41 741–749 and gag 77–85) and the new technique using rAAV infected autologous B cells or dendritic cells (DC) as stimulator. The results obtained from these 2 methods were compared.

Results: Frequency of CD3+/CD8+/IFN-γ+ cells was lower in the test using rAAV infected B cells than in the standard method using HIV specific CTL peptides. It might be due to the infection rate of rAAV was less than 3% in B cells. However, frequencies of CD3+/CD8+/IFN-γ+ cells were similar when compared results between the tests using rAAV infected DC or purified rAAV infected B cells for the stimulation and those tested by the standard method.

Conclusion: Theoretically, this may be used for detection of HIV specific CTL, but the infection rate of rAAV in B cells should be improved. Alternately, the other viral delivery system which has higher infection rate for B cells, such as lentivirus, may be used.

This study was supported in part by AIDS Trust Fund, Hong Kong.

P722 Neuropeptide S receptor 1 variation is associated with Chlamydia pneumoniae seropositivity in Finnish military conscripts

T. Laajanen, A. Rantala*, R. Jaanonen, T. Laitinen, M. Leinonen, J. Kere, P. Saikka (Oulu, Helsinki, FI; Stockholm, SE)

Objectives: Chlamydia pneumoniae is a common Gram-negative respiratory pathogen that has been suggested to associate e.g. with asthma. Neuropeptide S receptor 1 (NPSR1, GPR154, GPRAR) is a susceptibility gene for asthma and related phenotypes. NPSR1 is expressed in macrophages, and lipopolysaccharide (LPS) stimulation has been suggested to affect NPSR1 expression. Our aim was to study if NPSR1 polymorphisms associate with C. pneumoniae seropositivity.

Results: NPSR1 haplotypes H1 (frequency = 0.34) and H2 (frequency = 0.25) were associated with high C. pneumoniae IgG and/or IgA antibodies (IgG titre > 128 and/or IgA titre ≥40) both at entry (n = 144, 16%) and at end (n = 91, 79%, 12%) of the service. The infection rate for B cells, such as lentivirus, may be used.

The results of these 2 methods were compared.

Conclusion: Theoretically, this may be used for detection of HIV specific CTL, but the infection rate of rAAV in B cells should be improved. Alternately, the other viral delivery system which has higher infection rate for B cells, such as lentivirus, may be used.

This study was supported in part by AIDS Trust Fund, Hong Kong.

P722 IgG-antibody response against Clostridium difficile antigens, PCR-ribotype and relapse of C. difficile-infection

M.P. Bauer*, J.R. Poxton, K. Adamowicz, E.J. Kuijper, J.T. van Dissel (Leiden, NL; Edinburgh, UK)

Objectives: To investigate the IgG antibody response against C. difficile antigens in patients with C. difficile infection (CDI), and establish the correlation with infecting PCR-ribotype and previous episodes as well as future relapse of CDI.

Methods: In sera of patients with toxin assay-positive and culture-proven CDI who had completed 10 days of antibiotic therapy for CDI, IgG titres against EDTA extracts (cell surface molecules), guanidine hydrochloride extracts (surface-layer proteins) and culture supernatant (crude toxin) of ribotypes 001 and 027, and against lipoteichoic acid of ribotype 001, were determined by enzyme-linked immunosorbent assay (optical density [OD]). By multivariate analysis, we correlated the IgG titres with infecting ribotype (027 versus other), prior CDI episodes (first versus >1 prior episodes) and future relapse within a 60-day follow-up period.

Results: Patients suffering CDI caused by ribotype 027 had higher ODs against EDTA extracts and crude toxins of both ribotypes as compared with patients with CDI caused by other ribotypes. IgG antibody titres against antigens from ribotype 001 were higher than those against antigens from ribotype 027. Against EDTA extracts and crude toxins
of ribotype 27, the difference in ODs between those infected by O27 versus other ribotypes reached a level of significance. In all patients, ODs in ELISA measuring antibody titres against surface-layer proteins were low. Of note, IgG antibody titres were not influenced by number of prior CDI episodes nor a predictor for future relapse.  

Conclusion: Patients infected by *C. difficile* ribotype O27 have higher ODs, reflecting a higher concentration or higher affinity in the IgG-ELISA, against cell surface molecules and crude toxins as compared with patients infected by other ribotypes. IgG titres were not correlated with number of prior episodes, nor were they a predictor for future relapse.

**P724** The association of mannose-binding lectin with elevated body mass index

A. Ranta*1, T. Lajunen, J. Korvuo, P. Vikatmaa, P. Saikku, M. Leinonen (Oulu, Helsinki, FI)

Objectives: In previous studies obesity has been linked to inflammatory pathways. Mannose-binding lectin (MBL) is an important serum protein involved in innate immune defence system. MBL deficiency and MBL2 gene polymorphisms have been associated with decreased defence response and recurrent infections which may be associated to low-grade inflammation. A recent study found that MBL was associated with insulin resistance and obesity. Our aim was to study the association of serum MBL levels and MBL2 gene polymorphisms with overweight and obesity in patients with cardiovascular diseases.

Methods: Six single nucleotide polymorphisms in the promoter region (alleles H1, X1, Y1 and P1) and exon 1 region (variant alleles D, B and C and wild-type allele A) of the MBL2 gene were genotyped by a real-time PCR and serum MBL concentrations were measured by commercial ELISA test in 174 patients with symptomatic carotid stenosis, abdominal aortic aneurysm or occlusive aortic disease. Information about BMI and hypercholesterolaemia was available. SPSS15.0 was used for statistical analysis.

Results: BMI was divided into three groups: normal weight (BMI < 25.0), overweight (BMI 25.0−29.9) and obese (BMI ≥ 30.0). MBL2 exon 1 variant allele genotypes (A/O or O/O, where O indicates any of the variant alleles D, B or C) were more common among overweight and obese group (BMI ≥ 25.0) than among the normal weight group (38% vs. 20%; p = 0.021) and the risk was up to 2.4-fold (95% CI: 1.1−4.9) when adjusted for age, gender and the disease group. Also a borderline significant association was seen between the three BMI groups (20% vs. 38% vs. 35%; p = 0.066). In addition, exon 1 variant genotypes and possibly also an MBL level below the median concentration associated with hypercholesterolaemia (p = 0.008 and 0.071, respectively). MBL2 promoter polymorphisms were not associated with BMI or hypercholesterolaemia.

Conclusions: In this study, MBL2 structural variant genotypes significantly associated with BMI of ≥25 and hypercholesterolaemia which both are also connected to overweight. Since exon 1 variant alleles are strongly associated with MBL deficiency and recurrent infections in several studies, the theory on the link between obesity, MBL, infections and inflammation seems possible. However, an association between MBL levels and BMI was not detected here. Further studies are needed to replicate these findings.

**P725** A SNP in IFNγR1 promoter is correlated to the susceptibility to chronic HBV infection in Chinese population

Y. Zeng*, J. Zhou, D.Q. Chen, V.K.M. Poon, F. Ng, K.Y. Yuen, B.J. Zheng (Hong Kong, HK)

Objectives: The antiviral mechanism stimulated by interferon-gamma is found to be crucial for clearance of hepatitis B virus (HBV) in vivo. The antiviral signaling transduction is triggered by the specific binding between interferon-gamma and its receptor IFNGR1 (interferon-gamma receptor 1). Interferon-gamma signalling transduction pathway is directly controlled by the IFNγR1 expression level. In our study, single nucleotide polymorphisms (SNPs) in the IFNγR1 gene and the correlation between the SNPs and susceptibility to chronic HBV in Chinese were investigated.

Methods: Blood samples of 983 Chinese, including 361 chronic hepatitis B patients, 366 healthy individuals, and 256 hepatitis B spontaneously recovered patients, were collected. Seven SNPs (−611A/G, −56C/T, 40G/A, 95C/T, 130A/G, 20685A/G, 21227T/C) in IFNγR1 gene were identified by restriction fragment-length polymorphism (RFLP) assays. The transcription levels of different SNPs variants were compared by luciferase assays.

Results: −56C and −56T allele were found to be correlated to HBV clearance and persistence. In luciferase assays, the transcription level of IFNγR1 promoter with the −56C is significantly higher than that with −56T.

Conclusion: −56C/T SNP in IFNγR1 promoter region is associated with susceptibility to chronic HBV in Chinese population.

**P726** Comparison of two *Mycobacterium bovis Bacillus Calmette-Guerin* preparations used for immunotherapy of human superficial bladder cancer

W. Janaszek-Seydlitz*, M. Ratha, B. Buchole (Warsaw, PL)

Objectives: Aim of present study was comparison of safety and immunoreactivity of two *Bacillus Calmette-Guerin* (BCG) preparations used in Poland for bladder cancer immunotherapy.

Methods: BCG preparations. Onco – Tice (Organon Teknika, The Netherlands) preparation (Tice strain), Onko BCG 50 (Biomed Lublin, Poland) preparation (Brazilian Moreau strain). Bladder cancer cell line. T24, a transitional cell of bladder cancer cell line of human origin. Viability and thermostability. Classical technique for the determination of the number of CFU and thermostability of BCG preparations on a solid Ogawa medium have been used. Multiplication BCG in spleen of mice. Residual virulence of compared BCG substrains was evaluated on Balb/c mice injected intravenously. Attachment of BCG to bladder cancer cells. Cancer cells T24 were cultured together with BCG preparations. After 1, 2, 3, 4, 5 and 6 days of culture the total number of cancer cells and number of cancer cells with attached BCG were counted. Cytostatic effect of BCG. Antitumour activity was based on luminometric measuring of the ATP activity of viable cancer cells in suspension with or without presence of BCG preparations.

Results: Both BCG preparations showed the high number of viable culture forming units and good thermostability. The peak of bacillus multiplication in mice spleen was observed 2 weeks after i.v. injection of BCG dosage. It was 1.8 × 10⁶ CFU/spleen in mice vaccinated with Tice BCG and 1.1 × 10⁶ CFU/spleen in mice vaccinated with Moreau BCG substrain. The peak of attachment of BCG to T24 tumour cells occurred after 2 days of cultivation. The maximal percentage of the tumour cells with attached BCG was 56% for Onko BCG50 and 51.4% for Onco-Tice. BCG-inhibited T24 cell growth was dependent on concentration of BCG and time of cultivation. Both BCG substrains had similar profile of inhibitory effects.

Conclusion: Preparations Onco-Tice and Onko BCG 50 used for immunotherapy of superficial bladder cancer have been showed similar residual virulence and very similar viability and thermostability.

− The higher percentage of tumour cells with attached BCG showed the cultures with Onko BCG (Moreau).
− Both preparations showed similar profile of cytostatic activity.

**P727** Moxifloxacin decreases neutrophilic inflammation in the LPS-treated ferret airway

B.K. Rubin, M. Pearce* (Winston-Salem, US)

Objectives: Quinolone antibiotics concentrate in inflammatory cells and may be immunomodulatory. We hypothesised that oral moxifloxacin...
could ameliorate neutrophil-induced airway inflammation in ferret tracheae exposed to bacterial endotoxin (LPS).

**Methods:** Ferrets (N=32) were anaesthetised and intubated with an endotracheal tube coated with 10 mg of LPS for 30 minutes per day for 5 days. Starting at day 4 and continuing for five days, moxifloxacin 10 mg/kg was administered to 24 animals by nasogastric tube and saline control to 8. On day 9 and again on day 11, twelve moxifloxacin-treated ferrets and four control animals were sacrificed and the trachea excised. Mucociliary transport was measured by timing the movement of charcoal ash across a 3 mm segment of trachea. Attached mucin (µg/mg tissue) and lysozyme as well as secretion over 1 hour were measured in an additional tracheal segment. The integrity of the ciliated epithelium and infiltration of the epithelium by neutrophils were determined by light microscopy after haematoxylin and eosin plus Alcian blue/PAS staining.

**Results:** LPS-induced neutrophilic inflammation was associated with dose-dependent histological evidence of airway damage. There was significantly less neutrophil accumulation (Figure, P < 0.03) and a trend toward decreased mucin secretion (P = 0.07) in the moxifloxacin-treated animals.

**Conclusion:** Topically applied LPS induces neutrophil influx in the ferret trachea and is associated with mucin hypersecretion and epithelial metaplasia. These changes can be ameliorated by 5 days of moxifloxacin treatment.

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**Adenosine A2A receptor agonist (2-chloroadenosine)**

**Interaction with complement factor H and C4b-binding action of bacterial extracts from Klebsiella pneumoniae 5055-induced acute lung infection in BALB/c mice**

V. Kumar*, S. Chhibber (Chandigarh, IN)

**Objective:** Present study has been designed to evaluate the anti-inflammatory and immunomodulatory effect of adenosine A2 receptor analog called 2-chloroadenosine (2-CADO) in *Klebsiella pneumoniae* B5055 induced acute lung infection in mice.

**Methods:** The animals were divided into 2 groups: a. control and b. 2-Chloroadenosine (2-CADO) treated. Acute lung infection in mice was induced by directly instilling the selected dose (104 cfu) of bacteria intranasally. Histoptahological examination of lungs was done for assessing neutrophil infiltration and degree of lung inflammation. Besides that other inflammatory parameters myeloperoxidase (MPO), malondialdehyde (MDA), nitric oxide (NO), TNF-α, IL-1α, MPO, MDA and NO were also estimated in lung homogenate. The 2-chloroadenosine was administered intravenously at a dose of 10 µg/kg/day.

**Results:** The lungs of control group animals on histopathological examination revealed the profound neutrophil infiltration into the lung alveoli. TNF-α, IL-1α, MPO, MDA and NO were also significantly (p < 0.05) increased. However, intravenous treatment of animals with 2-CADO (10 µg/kg/day) significantly (p < 0.05) decreased the neutrophil infiltration into the lung alveoli without lowering the bacterial count in lungs. A significant (p < 0.05) decrease in TNF-α, IL-1α, MPO, MDA and NO along with elevation of IL-10 levels in lung homogenate of mice was observed upon treatment with 2-CADO.

**Conclusion:** A2A receptor agonist (2-CADO) protected acute lung inflammation via acting as an immunomodulatory agents in acute lung infection.

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**Interaction with complement factor H and C4b-binding protein contributes to the serum resistance of an anaerobic pathogen, Fusobacterium necrophorum**

N. Friberg*, P. Carlson, S. Meri, H. Jarva (Helsinky, FI)

**Background:** Anaerobic bacteria are part of our environment and flora. They usually become invasive and pathogenic only under special conditions e.g. tissue hypoxia, concomitant infection or neoplasia. *F. necrophorum* is an exception and can on its own be pathogenic. This strictly anaerobic Gram-negative rod is involved in local and invasive diseases such as the life-threatening Lemierre’s syndrome. This syndrome, characterised by sore throat, septicaemia, jugular vein thrombosis and disseminated infection, affects mainly otherwise healthy young adults. In order to cause disease, *F. necrophorum* must evade innate immune responses. Innate immunity is the first-line of defence against microorganisms. An important and efficient part of the innate immunity is the complement (C) system.

**Objectives:** The aim of our study was to understand how *F. necrophorum* avoids C activation and whether it binds C inhibitors such as factor H (FH) (inhibitor of alternative pathway) and C4 binding protein (C4BP) (inhibitor of the classical and lectin pathways).

**Methods:** We collected twelve *F. necrophorum* strains isolated from patients with sepsis. To study the serum resistance, strains were incubated in 75% serum and grown under anaerobic conditions. To detect binding of C inhibitors, we used radiolabeled proteins and flow cytometry.

**Results:** All strains were resistant to serum killing after a one-hour incubation. All strains bound FH, except strain 12. FH-binding was ionic in nature, specific and occurred via sites on both the N- and C-terminus. All strains bound C4BP. The interaction between *F. necrophorum* and C4BP was hydrophobic and specific in nature. Bound complement inhibitors remained functionally active as a cofactor for factor I in the cleavage of C3b for FH and C4b for C4BP. Strain 12 did not bind FH and bound less C4BP than the other strains. Its survival in normal human serum was impaired after 3.5 hours incubation compared to the other strains. Interestingly, patients with the most severe symptoms carried strains with the strongest ability to bind FH and C4BP. The carrier of strain 12 had not developed a typical Lemierre’s syndrome. This suggests that the binding of C inhibitors contributes to the virulence and the survival of *F. necrophorum* in the human host.

**Conclusions:** We show for the first time that an anaerobic bacterium is able to bind the C inhibitors FH and C4BP to evade C attack.

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**Action of bacterial extracts from Aggregatibacter actinomycetemcomitans in the production of IL-6 and IL-8 by human gingival fibroblasts**

O. Gonzalez, N. Roa, C. Valdivieso, F. Gamboa* (Bogota, CO)

**Objective:** Periodontal disease is refers to a pathological response that cause inflammation and loss of supporting structures of the teeth. *Aggregatibacter actinomycetemcomitans* is a Gram-negative bacillus associated with aggressive forms of periodontitis. This microorganism express a cytotoxical distending toxin (Cdt), which induces cell cycle arrest and modulates cytokine synthesis. The aim of this study was to evaluate the action of two bacterial extracts from *A. actinomycetemcomitans* (Cdt mutant and wild-type strains) in the production of IL-6 and IL-8 by human gingival fibroblasts (HGF).

**Methods:** The supernatants from HGF primary cell cultures were exposed to either wild type *A. actinomycetemcomitans* or Cdt mutant strain bacterial extracts. The IL-6 and IL-8 production was determined by using a cytometric bead array human inflammation kit (Becton-Dickinson).

**Results:** There were not significant differences in IL-6 and IL-8 levels produced by HGF challenged with wild type or Cdt mutant bacterial extracts. However, when response to lipopolysaccharide was not controlled by preincubation of HGF with antibodies against CD14 and anti-TLR4 before bacterial challenge, IL-8 production was significantly reduced in HGF stimulated with Cdt mutant but not with *A. actinomycetemcomitans* wild type strain.
Conclusion: These results suggest that Cdt does not affect directly the IL-6 and IL-8 production by HGF in vitro. Nevertheless, a potential indirect immunostimulatory role of Cdt in HGF may be associated with other *A. actinomycetemcomitans* bacterial components such as lipopolysaccharide, which has to be studied.

P732 Smoking status interacts in the association between mannose-binding lectin serum levels and carriage of *partialia* bacteria


Objective: It is known that carriage of respiratory bacteria is a major factor in the transmission of the infection and in some cases the infection can lead to respiratory or even systemic disease. Mannose-binding lectin (MBL) is an important molecule of innate immunity: it acts as an opsonin and stimulates the complement lectin pathway. The MBL insufficiency has been associated with increased susceptibility to common respiratory infections as well as invasive infections. We studied the association of smoking and MBL concentrations with oropharyngeal carriage of *S. pneumoniae, N. meningitidis, H. influenzae, M. catarrhalis* and beta-haemolytic streptococci in young men with and without asthma.

Methods: We measured MBL concentrations by a commercial ELISA test in 518 military conscripts (127 asthmatics and 391 controls) of Kajaani garrison. Oropharyngeal swabs were collected from study participants at the beginning, at the end of the military service and during all infectious episodes requiring consultation by the physician. The isolates were cultured and identified using generally accepted methods.

Results: The carriage rate of beta-haemolytic streptococci (*P* < 0.001), *S. pneumoniae* (*P* = 0.002) and *N. meningitidis* (*P* = 0.005) throughout the military service was significantly higher among smoking military conscripts than in non-smokers. In non-smokers, under median MBL level proved to be a significant risk factor for the carriage of beta-haemolytic streptococci (OR = 2.0; 95% CI 1.2–3.2), *N. meningitidis* (OR = 1.9; 95% CI 1.0–3.5) and a borderline significant risk factor for the carriage of *S. pneumoniae* (OR = 1.5; 95% CI 0.9–2.5). Same kind of association was not found in the smokers.

Conclusions: Non-smoking conscripts with low MBL concentrations are prone to acquisition of oropharyngeal carriage of beta-haemolytic streptococci, *N. meningitidis* and *S. pneumoniae* during military service.

P733 Regulation of immuno-pathological response in mouse lungs by TGF-β1 after influenza A virus infection

M. Khanna*, V. Sricastava, V. Vijuayan, R. Kalshrestha (New Delhi, IN)

TGF-β1 is a potent immunomodulator and regulates the inflammatory process in a complex biphasic fashion. The immune response to influenza A virus is characterised by an influx of both macrophages and lymphocytes into the lungs of the infected host. We hypothesize that the TGF-β1 negatively regulates the inflammatory response by regulating lymphocyte influx to the airway and further modulating the release of proinflammatory and anti-inflammatory cytokines. Eight-week-old BALB/c mice were intranasally instilled with influenza A virus (A/ بغداد/317/72/H3N2), 4.1×10^6 PFU of virus in 50 μl of allantoic fluid or mock infected 50 μl of allantoic fluid. TGF-β1 administered to mice by giving intravenous injection of rTGF-β1, 0.5 μg/Kg body weight of mouse. The mice were euthanised on days 3, 5 and 7 postinfection for the analysis of parameters. We observed an increase of lymphocyte count both on 3rd and 5th day p.i however administration of rTGF-β1 with virus reduced the lymphocyte count. Lungs of mice showed clear progression of the inflammation, significant alveolitis, with necrosis of epithelial cells. The alveoli, interstitial septa and perrivascular spaces were extensively infiltrated by a mixture of inflammatory cells on 3rd, 5th and 7th days after influenza virus infection. An increase of INF-γ level observed 3rd day of post infection however IL-10 level was maximum on 7th day and INF-γ level reduced to basal level on 7th day. Simultaneous administration of rTGF-β1 with virus instillation inhibited release of INF-γ level on third day and increased level of IL-10 level seventh day. rTGF-β1 acts as an immunomodulatory cytokine and inhibits lymphocyte influx after virus infection and lymphocyte activation. It modulates the inflammatory process by inhibiting INF-γ a proinflammatory cytokine and increased release of IL-10, which is an anti-inflammatory cytokine. rTGF-β1 affects recruitment of inflammatory cells at the site of inflammation by inhibiting lymphocyte invasion and interfering cytokines mediated inflammatory cascade by less involvement of lungs.

P734 Relevance of procalcitonin level on the serum lipopolysaccharide concentrations in intensive care unit bacteraemic and non-bacteraemic patients


Objective: Procalcitonin (PCT), in addition to being a pivotal marker of sepsis severity, has been reported to be a mediator of the systemic inflammatory response associated to life-threatening infection. Concentration of PCT increases in serum samples of septic shock patients until immunonannalysis. In experimental animals, co-administration of PCT with bacterial lipopolysaccharide (LPS) increases lethality, while immunonannalisation of PCT produced encouraging results for sepsis therapy. The aim of the study was to investigate the changes in the PCT, LPS and IL-10, as well as their relationship with severity of the clinical conditions among ICU patients stratified based on blood culture positivity.

Methods: The serum level of PCT was tested by Enzyme Linked Fluorescent Antibody (ELFA) procedure (Vidas B.R.A.H.M.S., PCT BioMerieux, Italy), LPS concentration by Limulus Amoebocyte Lysate (LAL) test (QCL-1000, Cambrex, Walkersville, USA), IL-10 was evaluated by ELISA (Bender MedSystems GmbH, Vienna, Austria) in samples obtained at the time of the blood culture (Bact/ALERT 3D, BioMerieux, Italy) and at different periods thereafter.

Results: In blood cultures positive patients, LPS concentrations at the zenit of PCT levels were significantly (*P* < 0.05) increased in comparison to LPS evaluated at the nadir of PCT concentration. On the contrary among blood culture negative patients, when PCT peaked the LPS levels were found significantly (*P* < 0.05) lower than LPS concentrations at the minimum of PCT levels. SOFA score and serum IL-10 were higher among bacteraemic patients versus culture negative subjects.

Conclusion: LPS was found a reliable marker of sepsis severity in the culture positive patients if evaluated together with PCT. The paradoxical feature, of a quite high LPS level when PCT was very low in nonbacteraemic subjects, deserves further investigations.

Pathogenesis of infections caused by Gram-positive bacteria

P735 Role of rsbU and Staphyloxanthin in intracellular growth of *Staphylococcus aureus* in human phagocytic cells (THP-1 macrophages)

A. Olivier, S. Lemaire, F. Van Bambeke, P. Tulkens*, E. Oldfield (Brussels, BE; Urbana, IL, US)

Background and Aims: *S. aureus* intracellular survival is critical for persistence of infection. rsbU expression stimulates the production of Staphyloxanthin (SFX), a yellow pigment that protects *S. aureus* against oxidant damage (Science [Wash.] 2008; 319:1391–1394). We have examined the role of rsbU and SFX in phagocytosis and growth of *S. aureus* in phagocytic cells.

Methods: Bacteria: strain 8325-1 (natural deletion in rsbU) and SH1000 (isogenic rsbU+ construct); USA300. Cells: THP-1 macrophages cultured and infected as previously described (AAC 2008; 52:797–805). Impairment of SFX synthesis: BPH-652 (3-phenoxy-alpha-phosphono-benzenesulphonic acid [dehydroquinolene inhibitor]).
**Results:** 8325–4 produced no SFX whereas SH1000 and USA300 were pigmented. BPH-652 impaired pigmentation of both SH1000 and USA300 at 1 μM in MH broth. The figure shows (A) that SH1000 was more resistant to inactivation by hydrogen peroxide; (B) that intracellular growth SH1000 and USA300 were more intense than that of 8325–4, and that addition of BPH (100 μM 48 h prior to phagocytosis [broth]) and during intracellular growth [culture medium]) reduced their growth to the level of that of 8325–4.

**Conclusion:** rsbU functionality and SFX production is an important factor in promoting intracellular growth of *S. aureus* in macrophages. This effect may be due to SFX-mediated resistance to oxidative stress. Inhibition of SFX synthesis may help in controlling intracellular *S. aureus* infection.

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**Staphylococcus aureus secretes two homologues Fc gamma receptor antagonists**

A.M. Steenbergen*, A. Kuipers, A.Y.L. Weersink, J.G.J. van de Winkel, K. van Kessel, J. van Strijp (Utrecht, NL)

**Objectives:** Fc gamma receptors (FcγR) play an essential role in the specific cellular defence against invading pathogenic bacteria, like *Staphylococcus aureus* (*S. aureus*). They are expressed on phagocytes and initiate phagocytosis in response to immunoglobulin G (IgG) coated on the bacterial surface. *S. aureus* evolved several mechanisms to evade host immunity. However, staphylococcal evasion molecules targeting FcγR have not been described yet. Our goal was to investigate whether *S. aureus* secretes any FcγR antagonists.

**Methods:** Culture supernatants of various clinical and laboratory *S. aureus* strains were screened for their ability to inhibit specific FcγR staining on phagocytes, analyzed by flow cytometry. An inhibitory protein was purified from one of the effective staphylococcal supernatants using d-lygand affinity chromatography, gel filtration, and FcγR coated magnetic beads. Subsequently the purified protein was identified by mass spectrometry. To determine its FcγR modulating capabilities recombinant protein was generated. Direct binding of the inhibitor to the different FcγR subclasses, its ability to block FcγR-IgG interaction and its effect on FcγR mediated phagocytosis and intracellular killing were analysed using FACS and ELISA.

**Results:** *S. aureus* is able to evade FcγR mediated immunity by secreting two potent FcγR antagonists, FLIPR and its homologue FLIPR-like. Both proteins bind to FcγR and block FcγR-IgG interaction. They inhibit FcγR mediated phagocytosis and intracellular killing of *S. aureus*.

**Conclusions:** Our findings increase the insight into the immune escape mechanisms of *S. aureus*, and furthermore might lead to the development of novel therapeutic agents in FcγR mediated diseases, like allergy and autoimmunity.

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**Biocide exposure activates sae promoter activity in *Staphylococcus aureus* strain Newman and increases cellular invasion**

D. Schäfer, T.T. Lam, T. Geiger, M. Mainiero, A. Bosscherhoff, M. Hussain, M. Bischoff, M. Frosch, S. Engelmann, C. Wolz, J. Reidl, B. Sinha* (Würzburg, Tubingen, Heidelberg, Munster, Homburg-Saar, Greifswald, DE; Graz, AT)

**Objectives:** *Staphylococcus aureus* has a high potential to cope with changing environmental conditions like heat, pH and chemicals. This is mostly due to its large number of global regulators such as the sae (*S. aureus* exoprotein expression) regulon, a two-component-like signalling system. Therefore we tested the ability of *S. aureus* strain Newman to react to sub-lethal concentrations of the commonly used biocide Perform® (Schülke & Mayr) and its major components.

**Methods:** Changes after exposure to Perform and its components were monitored by real-time RT-PCR, promoter activity assay, SDS-PAGE, biofilm assay and invasion assays (flow cytometry and lysostaphin protection).

**Results:** Perform or its component SDS induced a similarly altered protein pattern compared to untreated controls, as determined by SDS-PAGE. Most prominently, augmented bands were found for adhesins like Eap and Efb, which are known to be regulated by the sae regulon. Up-regulation of eap and sae (compared to 16S-rRNA) was confirmed by rtRT-PCR. A promoter activity assay of the sae promoter P1 showed an up to 140% increased P1 activity by treatment with Perform and SDS. In addition, *S. aureus* strain Newman exposed to Perform, but not SDS, showed a stronger biofilm formation. Perform and SDS enhanced cellular invasiveness to 250% and 320% of untreated controls, respectively. Increased invasiveness of Perform- and SDS-treated strain Newman was dependent on Eap and the sae regulon, but independent of agr, sarA and FnBPs, as determined by isogenic mutants. A sigB mutant had the tendency to further enhance cellular invasiveness.

**Conclusion:** Exposure of *S. aureus* strain Newman to sub-lethal concentrations of Perform or its component SDS activates the sae promoter P1 resulting in increased cellular invasiveness. By contrast, biofilm formation was only enhanced by Perform, but not by SDS. Changes in the protein expression pattern, including up-regulation of Eap, under the control of the sae regulon may account at least partially for these effects.

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**Phenotype-specific small non-protein-coding RNAs of *Staphylococcus aureus***


**Objectives:** The formation of phenotypic variants of *Staphylococcus aureus*, in particular of the small-colony variant (SCV) phenotype, is only partially understood. Non-protein-coding RNA (npcRNA) genes have been found to act as key regulatory players in response to environmental changes and host signals. So far, only few npcRNAs have been described in Gram-positive bacteria including staphylococci.

**Methods:** Since growth rate specific-expression was observed for bacterial npcRNA, we performed identification of npcRNA candidates using total RNA of *S. aureus* isolated from different growth stages of an isogenic clinical strain pair displaying the normal and the SCV phenotype. Total RNA was extracted and size-fractionated (from 10 to 500 nt) and two separate cDNA libraries were constructed. A total of 10,000 cDNA clones were randomly sequenced and analysed by BLASTN database search. Northern blot analyses were applied for conformation of the expression of novel npcRNAs candidates.

**Results:** Overall, 183 putative candidates for novel npcRNAs were identified. The expression of 34 of the identified npcRNAs was experimentally validated and confirmed by Northern blot analyses. Growth phase specific regulation was detected for 23 npcRNAs. Of particular interest, *S. aureus* phenotype-specific expression of six npcRNAs was found: Whereas five of the identified novel npcRNAs were specifically expressed only in the normal phenotype, expression of one of the novel npcRNA candidates was restricted to the SCV phenotype. Most of the novel npcRNAs were stage specific-regulated in SCVs with the majority being down regulated at the late growth of SCVs. In addition, several of the newly identified *S. aureus* npcRNAs exhibited relationship to *S. aureus* pathogenicity. Some of the experimentally verified npcRNAs were originated from pathogenicity islands indicating a putative role in the regulation of *S. aureus* virulence.

**Conclusion:** For the first time, a classification of npcRNAs based on their differential expression between the normal and the SCV phenotype of *S. aureus* was established. Thus, a role of npcRNAs in the regulation of the divergent phenotype-associated behaviour of *S. aureus* in the...
host environment might be assumed. Further studies are warranted to elucidate the potential functions of the novel npcRNAs and their impact on the pathogenesis of *S. aureus* infections.

**P739**  Holol- and apo-transferrins interfere with adherence to abiotic surfaces and with adhesion/invasion to HeLa cells in staphylococci


**Objectives:** *Staphylococcus aureus* and *Staphylococcus epidermidis* are the major cause of infections associated with implanted medical devices, such as intravascular catheters, prosthetic heart valves and orthopaedic devices. Colonisation on abiotic and biotic surfaces is often sustained by biofilm forming strains. Human natural defences can interfere with this virulence factor. In these experiments we investigated the effect of human apo-transferrin (apo-TF, the iron-free form of an iron-binding serum glycoprotein) and holotransferrin (holo-TF, the iron-saturated form) on biofilm formation by *S. aureus* and *S. epidermidis*. For *S. aureus* the effect of apo-TF and holo-TF on the adhesion/invasion ability on human cell lines was also studied.

**Methods:** We used two *S. epidermidis* strains (1 clinical isolate and ATCC35984 strain) and two *S. aureus* strains (1 clinical isolate and ATCC6538P strain). Apo-TF and holo-TF were used at concentrations starting from physiological (3–4 ng/ml) up to non-bacteriostatic and non-bactericidal. Bacterial biofilm formation was assessed by Christensen assay. *S. aureus* adhesion and invasion assays were performed in HeLa cell lines. Bacterial invasion was determined by numbering viable bacteria resistant to gentamicin treatment 1 h after infection. Bacterial adhesion was calculated as difference between total bacterial count and bacterial invasion count, 1 h after infection.

**Results:** Both transferrins (TFs) do not possess bacteriostatic and bactericidal effects. A strong reduction in biofilm formation with both TFs was obtained. In particular, the reduction in biofilm formation was higher with holo-TF rather than obtained with apo-TF. Both TFs exerted a significant reduction of *S. aureus* adhesion to and invasion of HeLa cells. SDS-PAGE and zymogram analyses are ongoing in order to compare surface protein profiles of treated and non treated samples.

**Conclusion:** Our results suggest that both forms of TF could be used for antibacterial adjuvant therapy in infection sustained by staphylococci to strongly reduce their virulence related to adhesion and invasion.

**P740**  Beta-lactams targeting septum formation increase Panton-Valentine leukaoinn expression by *Staphylococcus aureus*

O. Dumitrescu*, C. Badiou, Y. Benito, M. Bes, J. Eitenne, F. Vandenesch, G. Lina (Lyon, FR)

**Objectives:** *S. aureus* is a human pathogen producing a high number of virulence factors, one of them being the Panton-Valentine leukocidin (PVL) which is responsible of cutaneous abscesses but also of severe necrotising pneumonia. Previous reports showed that PVL expression may be modulated in vitro by beta-lactams such as oxacillin which increase PVL release when used at sub-inhibitory concentrations. In this work we studied the effect of several beta-lactam antibiotics of PVL release and we explored the mechanisms by which these antibiotics modulate PVL expression.

**Methods and Results:** We assayed for PVL production (by ELISA specific quantification and mRNA expression) of a PVL positive reference strain cultured in presence of sub-inhibitory concentrations of several beta-lactams. The beta-lactam tested were oxacillin (non-selective beta-lactam), but also four molecules having specific affinity for one of the four penicillin binding proteins (PBP) of *S. aureus*: imipenem (PBP1 selective), cefotaxim (PBP2 selective), cefaclor (PBP3 selective) and cefoxitin (PBP4 selective).

We obtained increased PVL release in the culture supernatant (2 to 3 fold) as well as increased PVLmRNA expression of the cellular pellet (20 to 50 fold) when cultures treated with either oxacillin or imipenem but not with cefotaxim, cefaclor or cefoxitin.

Ipenem targets specifically PBP1, therefore we explored PBP1 depletion effect on the modulation of PVL expression by using a Cadmium inducible pbp1 antisense RNA. We observed that bacteria cultured in presence of Cadmium expressed high level of pbp1 antisense RNA (up to 150 fold when compared to bacteria cultured without Cadmium) and also increased PVLmRNA (20 to 25 fold when compared to bacteria cultured without Cadmium).

**Discussion and Conclusion:** In this work we confirmed that beta-lactams, at sub-inhibitory concentrations, may modulate PVL expression by *S. aureus*. We showed for the first time that not all beta-lactams have the ability to modulate PVL release; only those inhibiting PBP1 lead to increased PVL expression. Moreover, we showed that PBP1 depletion induces PVL expression. PBP1 being an essential enzyme of the cell wall septum formation, our observations support the fact that antibiotics, by blocking the septum formation, might increase PVL expression. Based on our data, further studies are needed in order to clarify the mechanisms by which septum formation arrest may induce increased virulence in *S. aureus*.

**P743**  Sialic acid: a preventable signal for pneumococcal biofilm, colonisation and invasion of the host


**Objectives:** The correlation between carbohydrate availability, pneumococcal biofilm, nasopharyngeal colonisation and invasion has been analysed in order to investigate possible signals changing the host-microbe equilibrium.

**Methods:** A series of sugars were used to evaluate their impact on extent of pneumococcal biofilm formation in a mirotiter biofilm assay and in a carriage model in mice. Specificity of effects was controlled by competition experiments using structural sugar analogues both in the in vitro and in vivo assays.

**Results:** Out of a series of sugars only sialic acid (N-acetylneuraminic acid) enhanced pneumococcal biofilm formation in vitro, at concentrations similar to those of free sialic acid in human saliva. In a carriage model in mice intranasal inoculation of sialic acid significantly increased pneumococcal nasopharyngeal counts and instigated the translocation of pneumococci to the lungs. Both sialic acid dependent phenotypes could be competed by neuraminidase inhibitors DANA, zanamivir and oseltamivir.

**Conclusions:** The link between levels of free sialic acid on mucosal, colonisation and initiation of invasive disease shows how a host-derived molecule can influence a colonising microbe and highlights a molecular mechanism which explains the epidemiologic correlation between respiratory infections by neuraminidase bearing viruses and bacterial pneumonia. The data provide a new paradigm for the role of a host compound in infectious disease pointing to new treatment strategies.

**P744**  Cell replacement therapy for brain damage after bacterial meningitis: neuronal stem/progenitor cells migrate, differentiate and integrate in organotypic hippocampal slices injured after challenge with *Streptococcus pneumoniae*

S. Hofer*, G. GrandlerGrund, K. Oberson, A. Ducray, H.R. Widmer, S.L. Leib (Berne, CH)

**Objectives:** Bacterial meningitis (BM) causes life-long disabilities in up to 50% of the survivors. The underlying brain injury prominently affects the hippocampus, a brain region involved in learning and memory function. Hippocampal injury is characterised by apoptosis of immature neurons in the subgranular zone of the dentate gyrus (DG). Stem/progenitor cells are promising candidates for cell replacement therapies aimed at improving neurofunctional outcome after bacterial meningitis. Here, hippocampal slice cultures were challenged with live *Streptococcus pneumoniae* (SP) to induce apoptosis of immature neurons and subsequently neuronal stem/progenitor cells were evaluated for their potential to survive, differentiate and integrate in the injured hippocampus in vitro.

**Methods:** An in vitro system combining long-term organotypic hippocampal slice cultures from postnatal rats with embryonic
stem/progenitor cells from the subventricular zone was established. To induce apoptosis of developing neurons in the hippocampus, the brain damage pattern characteristic for BM, the slices were kept in partially nutrient-deprived medium and were exposed to live SP together with the antibiotics penicillin and streptomycin to cause bacterial killing and lysis. Stem/progenitor cells expressing green fluorescence protein (GFP) were expanded as neurospheres. Cells were then grafted into the hilus region of the DG in injured hippocampal slice cultures after challenge with SP and in control slices. The survival and integration of grafted cells was examined on cryosections of the slice cultures and the differentiation stage was assessed by immunohistochemistry.

Results: Histomorphologic analysis revealed neurite outgrowth and migration of subventricular derived stem/progenitor cells into the DG of hippocampal slices 7 days after engraftment. GFP-expressing neurosphere cells were able to differentiate and to mature into neurons.

Conclusion: Grafted embryonic derived stem/progenitor cells survive, migrate, differentiate and integrate into injured hippocampal slices with apoptotic cell damage due to challenge with SP. The transplantation of neurosphere derived stem/progenitor cells may hold promise for regenerative therapies aimed at repair of brain damage in patients suffering from neurofunctional sequelae after bacterial meningitis. 

**P743** Differential expression of enterococcal virulence-related genes: clinical versus food strains

A.R. Carlos*, T. Semedo-Lemsaddek, M.T. Barreto Crespo, R. Tenreiro (Lisbon, Oeiras, PT)

Objectives: Enterococci are Gram-positive bacteria that have been associated both with health and illness. Understanding how these microorganisms become pathogens and the characteristics involved in such process might help preventing more severe diseases. In the present investigation the differential expression of eight virulence-related genes (clyM, clyB, clyA, clyl, agg, esp, efaAfm and efaAfS) was assessed for six clinical, eight food and two reference enterococci. Expression analysis was performed after enterococcal growth in media simulating infection sites (serum, BHI and urine) and environmental colonisation (skim milk). The effect of different temperatures (30 and 37ºC), infection sites (serum, BHI and urine) and environmental colonisation (skim milk). The effect of different temperatures (30 and 37ºC), osmolarities (0%, 2.5%, 5.0% and 6.5%) and pH values (6.0, 7.0 and 7.4) on gene expression was also assessed.

Methods: For all strains and conditions gene expression analysis was accomplished using Reverse-Transcriptase PCR followed by agarose gel electrophoresis. The expression of all virulence genes was normalised using the housekeeping genes 16SrDNA and rpoA.

Results: The expression level of the virulence-related genes analysed varied significantly considering all the conditions assayed (P<0.05).

Semen was one of the media that highly promoted an increase in gene expression. Skim milk was also found to stimulate gene expression, but to a lesser extent. Osmolarity showed a small effect on the expression of virulence-related genes while both pH and temperature appear to be important cues for gene expression with pH 6.0 and 37ºC greatly stimulating gene expression. While some of the strains under analysis appear to activate gene expression only in response to a particular condition, such as the clinical strain MMH594, other strains, (e.g. LN11, a food strain), appear to activate their expression machinery regardless the growth environment. However, no significant differences were detected when comparing the expression profiles of clinical and food strains (P>0.05).

Conclusion: The expression profiles obtained in the present investigation were found to be environmental and strain-dependent, since no constant response was observed neither for clinical nor for food enterococci. These results reinforce the need for a careful evaluation regarding the pathogenic potential of enterococcal strains.

**P744** Comparison effect of vitamin C vaginal tablet with metronida- zole vaginal gel treatment and relapse of bacterial vaginosis

Z. Abbaspoor* (Ahvaz, IR)

Introduction: Bacterial vaginosis is the common cause of abnormal vaginal discharge among women of reproductive ages.
**P746** Community and non-community-acquired bacteraemia: correlation between empiric antimicrobial therapy and susceptibility of micro-organisms isolated during 2007 in the Ile de France Microbiologists Network


**Objectives:** The aim of the study was to analyze the antimicrobial therapy administered before the first positive blood culture result and the antibiotic susceptibility of the isolates

**Methods:** During 2007 in the 8 hospitals of the Ile de France network, empiric antimicrobial therapy was reported for all patients with a positive blood culture. Antibiotic susceptibility was determined according to the recommendations of the French Microbiology Society. The therapy was defined as “appropriate” if the patient received at least one antibiotic the micro-organism was susceptible to. This definition was not based on clinical efficacy. The bacteraemia was community acquired or not according to clinical and anamnestic features.

**Results:** On the 2013 bacteraemia, 58.3% were community-acquired and 41.7% were non-community-acquired infections. Antimicrobial therapy was appropriate in 1286 (64%) cases, inappropriate in 266 (13%), and no antibiotic was administered in 461 (23%). In these first two cases, large spectrum antibiotics were most frequently used: third generation cephalosporins (25%), aminoglycosides (13–19%), fluoroquinolones (15–16%). Therapy was more frequently appropriate if the bacteraemia was community-acquired, was monomicrobial (Streptococci, Pneumococcus, or Escherichia coli), was associated with urinary or respiratory tract infection and if it occurred in paediatrics or maternity patients. Among non-community-acquired bacteraemia, appropriate therapy rate was almost the same for mono or polymicrobial bacteraemia, higher for Enterobacteriaceae and Streptococci and lower for Pseudomonas aeruginosa and Staphylococci bacteraemia.

**Conclusions:** Empirc antimicrobial therapy before blood cultures results was not prescribed in 23% of episodes. Those patients might have nor sepsis nor severe clinical features. Therapy of non-community-acquired bacteraemia might require multidisciplinary approach, while national recommendations were enough to take care of patients with community bacteraemia.

**Results:** For the CE population, clinical cure rates were 70.4% (133/189) for TGC versus 74.3% (139/187) for CTX plus MET (95% CI −13.1, 5.1; p = 0.009 for non-inferiority). Clinical response for subjects with APACHE II scores >10 were 56.8% (21/37) for TGC versus 58.3% (21/36) for CTX plus MET. Microbiologic efficacy was similar between the two treatment arms with 68.1% (94/138) of TGC treated organisms and 71.5% (98/137) of CTX plus MET treated organisms considered eradicated at the TOC. The most frequently reported adverse events (AE) were nausea 38.6% and vomiting 23.3% in the TGC subjects and 28.6% and 18.2%, respectively, in the CTX plus MET subjects. Overall discontinuation rates due to AE were slightly higher in TGC subjects than comparator subjects, 8.9% and 4.8%, respectively.

**Conclusion:** Tigecycline monotherapy was found to be non-inferior to a combination regimen of CTX plus MET in subjects with complicated intra-abdominal infections.

**P748** Tigecycline use in nosocomial osteomyelitis


**Objective:** New antimicrobial agents are urgently needed for clinical use due to the increasing prevalence and spread of multidrug-resistant bacteria. Specifically, multidrug resistant Gram-negative bacteria containing expanded spectrum betalactamases (ESBL), commonly found in hospitals, has severely limited the number of antimicrobial agents available for use. Tigecycline is a first-in-class glycylcycline antibiotic, and is indicated for the treatment of complicated skin and intra-abdominal infections caused by susceptible microorganisms. In osteomyelitis, this antibiotic is not indicated nor has such use been systematically evaluated in human studies. The authors describe 10 cases of osteomyelitis that tigecycline has been used to treat multidrug-resistant bacteria.

**Methods:** Report of 10 cases of nosocomial osteomyelitis due to ESBL Escherichia coli and Klebsiella pneumoniae infection. In all cases, the diagnosis had been done based on the clinical findings, imaging procedures and data from laboratory tests: leukocytosis, elevations in the erythrocyte sedimentation rate, and C-reactive protein level. Blood cultures were negative, but bone cultures were positive in six samples for ESBL E. coli (three from lumbar spinal column biopsy and three from sacrum biopsy) and four samples of K. Pneumoniae (two from tibia biopsy and two from femur biopsy). All 10 patients had allergic reactions due to the use of carbapenems, and treatments were changed to tigecycline intravenous (100 mg initial dose, then 50 mg every 12 hours).

**Results:** In the first week of treatment, clinical and laboratorial improve were evident. After 14 days of treatment, all the patients had negative bone cultures. The use of tigecycline was extended for more 46 days to complete treatment.

**Conclusion:** Although tigecycline is not indicated for the treatment of osteomyelitis and the duration of therapy is for a maximum of 28 days, the authors report ten cases of successful use for a longer period in osteomyelitis. We suggest that this antibiotic can be an option for ESBL treatment in patients that carbapenems can not be use.

**P749** Macrolide use in intensive care units

E. Meyer*, P. Gastmeier, B. Schroeren-Boersch, F. Schwab (Berlin, DE)

**Objectives:** To analyse macrolide use in German intensive care units (ICUs) participating in the SARI (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units).

**Methods:** Prospective unit based surveillance in 43 German ICUs from 2006 to 2007 (16 interdisciplinary, 9 medical and 18 surgical ICUs). Monthly data on antimicrobial use were obtained from the computerised pharmacy database. Consumption i.e. antimicrobial usage density (AD) was expressed as daily defined doses (DDD) and normalised per 1000 patient-days (pd).

**Results:** In 2006 and 2007, 43 ICUs reported data on 361,824 pd and the pooled mean antibiotic consumption of 1213 DDD/1000 pd without sulfactam. The median macrolide use was 102 (range 2−157) in interdisciplinary ICUs, 99 in medical ICUs (range 47−345) and 40...
in surgical ICUs (range 1–150). Median erythromycin use accounted for 52% of total macrolide use in interdisciplinary ICUs, 26% in medical, but 80% in surgical ICUs. The median percentage of macrolides on total antibiotic use was 9% (range 0–13) in interdisciplinary, 7% (range 4–24) in medical and 3% (range 0–12) in surgical ICUs.

Conclusion: Erythromycin is not only used as an antibiotic but also as a systemicacting prokinetic drug e.g. to treat postoperative adynamic ileus in patients undergoingabdominal surgery. However, recent Cochrane reports stated that erythromycin showed "homogenous and consistent absence of effect" as prokinetic drug. Therefore, it seems most worthwhile to revise the indication of erythromycin use especially in surgical ICUs all the more macrolide use accounted considerably to total antibiotic use in surgical ICUs (up to 12%). Furthermore, macrolide use is a known risk factor for MRSA.

**P750 Intravenous fosfomycin for the treatment of nosocomial infections due to carbapenem-resistant Klebsiella pneumoniae in critically ill patients. A prospective evaluation**

A. Michalopoulos, S. Virtsili, P. Rafaillidis, G.H. Halevelakis, M. Falagas * (Athens, GR)

Objectives: Nowadays ICU-acquired infections due to MDR Gram-negative pathogens remain a serious problem in critically ill patients. We examined the safety and effectiveness of fosfomycin in adult patients with ICU-acquired infections due to carbapenem-resistant Klebsiella pneumoniae (K. pneumoniae).

Methods: To assess the safety and effectiveness of intravenous fosfomycin as an adjunctive to the intravenous antimicrobial therapy for the treatment of life-threatening infections due to carbapenem-resistant K. pneumoniae, we prospectively examined all patients, who received intravenous fosfomycin.

Results: Fosfomycin was administered intravenously in 10 critically ill patients of mean age 67.3±15.2 years old (6 females) for treatment of hospital-acquired infections due to carbapenem-resistant K. pneumoniae. Mean APACHE II score on ICU admission was 23.1. Fosfomycin (2–4 gr. every 6 hours) was administered in combination with colistin in 6 patients, or gentamicin in 3 patients. Patients received fosfomycin for bacteraemia (n=2), VAP and bacteraemia (n=2), VAP plus urine tract infection (UTI) (n=2), UTI (n=2), bacteraemia and wound infection (n=1), and wound infection (n=1). The median time for the development of infection due to carbapenem-resistant K. pneumoniae was 29.5 days following admission to the hospital. The majority of patients developed multiple nosocomial infections mainly due to MDR Gram negative bacteria prior to this episode. The mean (± SD) duration of treatment was 14 (±5.6 days). All patients had good bacteriological and clinical outcome of infection. All-cause hospital mortality was 2/10 (20%); both patients died later on because of septic shock of other cause and multiple organ failure. Patients had prolonged ICU length of stay (median LOS = 34 days) and hospital stay (median LOS = 87.5 days). No patient developed adverse events related to the administration of fosfomycin.

Conclusion: Intravenous fosfomycin may be a beneficial and safe adjunctive treatment in the management of life-threatening ICU-acquired infections due to carbapenem-resistant K. pneumoniae in critically ill patients.

**P751 Relationship between piperacillin-tazobactam consumption and bacterial resistance in Colombian hospitals. A time-series analysis, 2004–2007**


Objectives: Determine the relationship between the piperacillin-tazobactam (TZP) consumption and resistance of Pseudomonas aeruginosa and Klebsiella pneumoniae to this antibiotic in three high complexity hospitals from Colombia.

Methods: Ecological time series study to relate the effect of TZP consumption in P. aeruginosa and K. pneumoniae resistance. Monthly hospital consumption of TZP was collected in three hospitals of two cities in Colombia (Bogota and Cucuta). Antibiotic consumption was grouped as the number of defined daily doses (DDD) per 100 bed-days (World Health Organization) each month between January 2004 and December 2007 (48 periods). P. aeruginosa and K. pneumoniae resistance to TZP was determined as the proportion of resistant isolates reported by hospital laboratory, data was analyzed in Whonet 5.4° (Geneva, WHO). Resistance proportions were translated to odds and log transformed (ln[R(1− R)]). Univariate monthly series per hospital were performed for consumption and resistance (ARIMA – Box Jenkins). The relationship between both variables was explored using transfer function models per institution. All time series analysis were done in SCA (Villa Park, USA).

Results: Average monthly consumption of TZP was 1.36 DDD per 100 bed-days. In the three hospitals there was an upward trend in TZP consumption for study period. There was an absolute increase of 0.56, 1.81 and 3.33 DDD per 100 bed-days for each hospital. Monthly average resistance proportion in each hospital was 13.9, 19 and 36.8% for K. pneumoniae and 4.7, 24.4 and 37.1% for P. aeruginosa. Univariate consumption analysis shows an increase. Transfer function in the six models evidence consistency between the statistical association of TZP consumption and the expression of resistance to this antibiotic for K. pneumoniae and P. aeruginosa (Figure 1).

Conclusions: There is consistent relationship between TZP consumption and resistance of K. pneumoniae and P. aeruginosa to this drug. TZP consumption can explain until 20% of resistance found in P. aeruginosa and 12% in K. pneumoniae. Remaining unexplained resistance can be related to other uncontrolled factors not considered in the models.
Results: Ten hospitals at the end of the observation period notified antimicrobial consumption information. There is an important decrease in general use of ciprofloxacin and the reject of ceftazidime use from 2004. An upward trend can be observed and predicted for ampicillin/sulbactam, ceftriaxone, meropenem, piperacillin/tazobactam and vancomycin. The rising in meropenem use is set in contrast with imipenem steady trend. Trends of some antibiotics are shown graphically in Figure 1.

Conclusions: antimicrobial consumption surveillance is a main step in antimicrobial stewardship programs design. The Nosocomial antibiotic use patterns could be reflecting the effect of rational use strategies in studied hospitals, microbiological profiles and antimicrobial availability conditions. This first report of antimicrobial consumption in hospitals in our country establishes the need for further research in the antimicrobial use and the relationship with antimicrobial resistance and the identification of determinants to direct control strategies.

Results: Ten hospitals at the end of the observation period notified antimicrobial consumption information. There is an important decrease in general use of ciprofloxacin and the reject of ceftazidime use from 2004. An upward trend can be observed and predicted for ampicillin/sulbactam, ceftriaxone, meropenem, piperacillin/tazobactam and vancomycin. The rising in meropenem use is set in contrast with imipenem steady trend. Trends of some antibiotics are shown graphically in Figure 1. The Antimicrobial consumption trends in DDD per 100 bed-days. Colombia, 2002−2007.

P753 Need for dose adjustment in hospitalised patients using intravenous antibiotics in a university hospital

J.A. Cortes*, J.Y. Rodriguez (Bogota, CO)

Objective: To evaluate the need for dose change of antibiotics among hospitalised patients according to pharmacodynamic targets.

Methods: Antibiotic use for IV administration was reviewed in every admitted patient to a new university hospital. In those in which serum creatinine levels were available the creatinine clearance (CrCl) was calculated, as was the need for dose adjustment. Therapeutic objectives were determined according to pharmacodynamic (PD) targets (β-lactams time > MIC; penicillins >50%, cephaprenem >65%, carbapenems >40%; quinolones AUC/MIC > 100; amnoglycosides Cmax/MIC > 10). MIC90 for the most frequently found microorganisms (example: S. pneumoniae for community pneumonia) were used in the PK/PD calculations. In those not reaching a PD target, the percentage of dose increase was also calculated.

Results: During the two first months of the new university hospital, 234 antibiotic formulations were reviewed, for 117 hospitalised patients, 55.2% of them were women. The mean age was 64.5 years-old (range 16−95). The most frequent diagnosis were urinary tract infection (30.7%), followed by skin and soft tissue infections (20.3%), community acquired pneumonia (17.7%) and COPD exacerbations (10.8%). 71.3% of the patients with antibiotic formulations had a creatinine level and CrCl was calculated. The mean CrCl was 65.6 ml/min (range 10−127).

10.7% of the patients had a CrCl lower than 30 ml/min and 28.1% had a CrCl lower than 50 ml/min.

Conclusions: Dose adjustments for renal impairment is frequently required, specially among older patients. Stewardship programs for correct antibiotic use might contribute to a better administration of IV antibiotics in older patients, specially if PD targets are used.

P754 Ten years of antibiotic consumption in ambulatory care: trends in prescribing practice and antibiotic resistance in Austria

S. Metz-Gercek*, A. Maieron, R. Strauß, P. Wieninger, P. Apfaltermayer (Linz, AT)

Objective: The primary aims of this study were to determine (i) the quantity and pattern of antibiotic use in Austria between 1998 and 2007, (ii) to analyze antibiotic resistance rates in relation to antibiotic consumption in important clinical situations in order to provide data for empiric therapeutic regimens in key indications.

Methods: Consumption data and resistance data were obtained via the Austrian European Antimicrobial Resistance Surveillance System (EARSS) and the European Surveillance on Antimicrobial Consumption (ESAC). The Anatomical therapeutic chemical (ATC) classification and the defined daily dose (DDD) measurement units were assigned to the data. DDDS and the number of packages (PID) were used to calculate the amount of antibiotic consumption. Antibiotic resistance was expressed in resistance rates being the percentage of resistant isolates compared to all isolates of one bacterial species.

Results: The overall antibiotic consumption measured in DDDS showed an increase of 10% between 1998 and 2007 whereas in PIDs a decrease of 3% was found. The consumption for substances within the drug utilisation 90% segment measured in PID increased for ciprofloxacin (+118.9%), clindamycin (+76.3%), oxacillin (−22.5%), minocycline (−21.9%) and clarithromycin (−9.9%). Since 2001, an increase in the percentage of resistant invasive E. coli isolates for amnopenicillins (from 35% to 53%), fluoroquinolones (from 7% to 25.5%) and 3rd generation cephalosporins (from 0% to 8.8%) was observed. The percentage of pneumococcal isolates (Pn) non- or intermediate susceptible to pen remained stable over the years at around 5%. In macrolides the rate of resistant isolates increased from 5% to 12.8% with a peak in 2005 at 14.7%.

Conclusions: The Austrian resistance data do not explain the change in prescribing practice. The increased use of ciprofloxacin has most likely contributed to rising resistance rates in E. coli. Because of very low levels of resistance against penicillin in spn there is no need for the application of broadspectrum pen, pen combination products (amoxycillin/clavulanic acid), third generation cephalosporins as well as new fluoroquinolones for the treatment of community acquired pneumonia in ambulatory care patients.

P755 Theory of planned behaviour and its use in antibiotic prescribing in a hospital setting

P. Cortoos*, W.E. Peertmans, B. Schreurs, K. De Witte, G. Laekeman (Leuven, BE)

Background and Objective: In order to understand barriers against antibiotic guideline use, qualitative methods have proven to be efficient.
However, they do not provide quantitative data allowing to track the most influential problems. The Theory of Planned Behaviour (TPB) describes the influence and relative importance of barriers on intention and behaviour. In this model, intention is moderated by Attitude against the behaviour, how it is perceived by others (Subjective Norm (SN)) and the ability to perform the behaviour (Perceived Behavioural Control (PBC)). We present the development and testing of a TPB-based questionnaire aimed to improve use of antibiotic guidelines.

**Design:** Barriers against guideline use were identified by focus group discussion (Cortoos, 2008) and were sorted in 3 categories: Attitude (What do you believe are the disadvantages of using AB guidelines?); SN (Are there any individuals or groups that would disapprove your AB guideline use?); PBC (What factors enable/prevent using the AB guidelines?). Sorting was done by 2 independent researchers. Within each category, barriers with highest quotation frequency were used for the questionnaire (Francis, 2004). Every category was completed with 4–6 generic questions, directly measuring the specific category. To test the influence of habit, a 12-item questionnaire was also included (Verplanken, 2003). Intention towards improved guideline use was measured with 3 generic questions. Actual use of antibiotic guidelines (behaviour) was measured on a scale between 0 and 10. All other questions used a 7-point Likert scale. The questionnaire was piloted within 100–150 patients and 10 randomly selected physicians with various experience and professional status.

**Setting:** tertiary care university hospital. This study is part of a larger project on guideline compliance.

**Results:** No major remarks were made on the questionnaire. A limited multivariate analysis showed a good fit ($R^2=0.865$; $P<0.001$) with significant influence of habit ($P<0.001$) on intended use of antibiotic guidelines with a smaller influence of PBC ($P=0.014$). SN and Attitude were not significant.

**Conclusions:** As a result of this pilot study, the questionnaire will be used on a larger scale in different hospitals. Preliminary analysis shows a major influence of habit on intended use of antibiotic guidelines combined with a modest influence of external factors. Attitude and supervisors or peers appear to be of low influence.

**P756 Influence of a restriction model on the rational use of antibiotics in a large, secondary care hospital**

A.M. van den Abeele, M. Baert, D. Rijckaert, D. Ommeslag* (Ghent, BE)

**Objectives:** Judicious use of antibiotics for resistant Gram positive organisms is difficult to organise. We present 5-year data (2003–2007) concerning therapeutic indications, posology, monitoring and side effects of vancomycin (VAN) and linezolid (Lzd) among all patients treated in our hospital.

**Methods:** Sint-Lucas hospital (816 beds) has a multidisciplinary, antibiotic restriction model in use, organised by the local antibiotic management team (AMT). For over 5 years, selective delivery of VAN and Lzd by hospital pharmacy is possible only after clinical and microbiological consultation, available on daily base and combined with (daily) therapeutic monitoring and dose adjustments. Methicillin-resistant Staphylococcus aureus (MRSA) or coagulase negative staphylococci (MRSE) infections are predominantly caused by catheter-related (CVC) or neutropenic septic episodes, osteomyelitis, postoperative meningitis and endocarditis. These infections are registered continuously and matched with usage of VAN and Lzd.

**Results:** Over 80% of all patients have documented MRSA/MRSE infection. Over 5 years the number of treated patients with VAN is stable (n=1100/year). Underdosage of VAN is frequent only in haemodialysis patients. Overdosing with serious side effects seldom occurs. Consumption of Lzd is growing (n=5 in 2003 to n=45 in 2007). Over 65% of Lzd treated patients have received VAN first, in 20% Lzd was first choice because of renal insufficiency or CVC problems. Duration of treatment with Lzd in different indications was not standardised with treatment times between 5 and 70 days.

**Conclusions:** The concept of a restriction model for antibiotics helps to achieve therapeutic levels of VAN and minimises side effects of VAN and Lzd in the individual patient. It also contributes to tailor local therapeutic guidelines. Duration of treatment for several indications has to be improved.

**P757 Prevalence of antibiotic use in hospitals in Cyprus**

E. Kritsotakis, E. Vounou, M. Kontou, P. Papakyriakou, M. Kolion-Mazeri, I. Dimitriadis, A. Gilas* (Heraklion, GR; Limassol, Nicosia, Pafos, Larnaca, CY)

**Objectives:** There are few data on antibiotic prescribing within Cypriot hospitals. This study, set out as part of a larger study of nosocomial infections, aimed to obtain main indicators of hospital antibiotic use in Cyprus.

**Methods:** A point prevalence survey was conducted in the 5 public hospitals of Cyprus in November 2006. The survey included all inpatients older than 1 year who, on the study day, had been present for at least 24 hours in the hospital. Data collected for all patients included demographics, antibiotics for systemic use received on the survey day and duration of prescription.

**Results:** On the survey day, 345 out of the 705 screened patients (48.9%) were receiving antibiotics (interhospital range: 38.8–53.3%), of whom 115 patients (33.3%) were receiving combination therapy. The highest prevalence of antibiotic use was observed in surgical wards (59.9%), followed by paediatric wards (54.2%), intensive care units (50%), medical wards (42.8%), and gynaecology-obstetrics wards (20.6%). A total of 176 patients (51.0%) received empirical treatment; 119 patients (34.5%) received surgical prophylaxis, and 30 patients (8.7%) received antibiotics for bacteriologically documented infection. For 20 patients (5.8%), no justification for antibiotic use was provided. Of the patients who received perioperative prophylaxis, 82 patients (68.9%) had undergone surgical operations classified as “clean”. The median duration of perioperative prophylaxis was 3 days (interhospital range: 2.0–3.5 days). Out of the total 468 antibiotics prescribed, the most commonly used classes included third generation cephalosporins (23.5%), second generation cephalosporins (17.9%), imidazoles (11.5%), fluoroquinolones (10.3%), carbapenems (8.1%), macrolides (5.3%), and glycopeptides (5.1%).

**Conclusion:** A high prevalence of hospital antibiotic use was found in Cyprus, compared to the prevalence seen in other European countries. The frequent use of combination therapy and broad spectrum antibiotics, their use in clean surgery and the extended duration of surgical prophylaxis observed in this study, are indicative of the potential for limiting and improving prescribing. Study data emphasize the need to develop effective antibiotic surveillance and management programmes in Cypriot hospitals.

**P758 Antimicrobial prescribing awareness campaign in the emergency department**

S. Foley*, F. Fitzpatrick, B. Masake, E. Smyth, H. Humphreys (Dublin, IE)

**Background and Objectives:** Antibiotics are frequently prescribed in the Emergency Department (ED) and continued for the duration of a patient’s admission. Doctors in the ED change every 6 months and frequently come from other hospitals. A September 2007 audit revealed that ED doctors prescribed the majority (68.6%) of antibiotics. 44% did not comply with hospital prescribing guidelines and microbiological investigations were not performed in 24% of patients. As the initial prescription and investigations can affect a patient’s outcome, a hospital wide programme was commenced targeting prescribers, specifically in ED.

**Methods:** A ‘quick reference’ antimicrobial prescribing guide was created, distributed to all doctors and placed on the hospital intranet homepage. Antimicrobial prescribing awareness education sessions were held for ED doctors. Weekly five minute educational sessions were performed by the microbiology team at medical grand rounds. An antimicrobial restriction list was put in place. The ED audit was repeated in September 2008.
A one-day prevalence study: the evaluation of antibiotic use and cost in a training hospital
A. Inan*, O. Ozturk, N. Ceran, S. Senbayrak, I. Erdem, P. Goktas
(Istanbul, Tekirdag, TR)

Purpose: Rational antimicrobial use is important not only for the effectiveness of the treatment but also to prevent spread of antimicrobial resistance and to decrease unwanted side effects and high costs. The aim of this study was to determine the usage patterns of antibiotics and cost of antibiotic therapy in hospitalised patients.

Methods: This one-day, cross-sectional study was conducted in Haydarpasa Numune Hospital, a 750-bed training and research hospital in Istanbul. On December 30, 2008 each hospitalised patient on medical and surgical wards was visited particularly by an infectious diseases specialist, and in the patients who received antibiotic, data concerning patient and antibiotic therapy were recorded. Statistical analysis was made Fisher’s exact test and the cost of antibiotic therapy was calculated as United States Dollars.

Results: On the study day 542 inpatients were evaluated. Antibiotic usage rate was 40.4% in all hospitalised patients and it was 36.9% and 44.3% in the patients on the surgical and medical wards, respectively (p<0.001). The most frequently used antibiotic was ampicillin–sulbactam in medical wards and cefazolin in surgical wards. The total empirical antibiotic use was more frequent (48.4%) than prophylactic (29.6%) and specific (based on culture result, 21.9%) use. The 23% of the antibiotic use in inappropriate and these prescribed antibiotics don’t need Infectious Disease Specialist’s approval. The total one-day cost of antibiotic therapy in our hospital was 5496 dollars, the mean daily cost per patient was 2.3 dollars for prophylaxis, 15.3 dollars for community acquired infections and 99.5 dollars for hospital infections.

Table 1. Antibiotic usage rates in Haydarpasa Numune Teaching Hospital

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Medical wards</th>
<th>Surgical wards</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>57</td>
<td>19.5</td>
<td>58</td>
</tr>
<tr>
<td>Cefotaxin</td>
<td>6</td>
<td>2.0</td>
<td>122</td>
</tr>
<tr>
<td>Ceftriazone and cefotaxine</td>
<td>41</td>
<td>13.9</td>
<td>44</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>9</td>
<td>3.1</td>
<td>9</td>
</tr>
<tr>
<td>Cefuroxime-sulbactam</td>
<td>22</td>
<td>7.4</td>
<td>4</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>46</td>
<td>15.6</td>
<td>28</td>
</tr>
<tr>
<td>Imipenem/cilastatin and meropenem</td>
<td>41</td>
<td>13.9</td>
<td>14</td>
</tr>
<tr>
<td>Ticlofloxacin and trovacin</td>
<td>25</td>
<td>8.4</td>
<td>4</td>
</tr>
<tr>
<td>Linezolid</td>
<td>4</td>
<td>1.5</td>
<td>5</td>
</tr>
<tr>
<td>Gentamicin and amikacin</td>
<td>5</td>
<td>1.7</td>
<td>23</td>
</tr>
<tr>
<td>Ciprofloxine and levofloxazine</td>
<td>11</td>
<td>3.7</td>
<td>4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>13</td>
<td>4.6</td>
<td>44</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>5</td>
<td>1.7</td>
<td>7</td>
</tr>
<tr>
<td>Other antibiotics</td>
<td>11</td>
<td>3.7</td>
<td>4</td>
</tr>
</tbody>
</table>

Total: 108 100.0 152 100.0 250 100.0

Conclusion: This study showed that the antibiotic usage rate was 40.4%, inappropriate usage rate was 23%, the total one-day cost of antibiotic therapy was 5496 dollars, and the total daily cost for hospital infection was 3583 dollars in our hospital. The antibiotic cost of hospital infections is an important part of extra costs that should be reduced providing effective infections control strategies and rational antibiotic usage in hospitals.

P759

Antibiotic consumption in Lithuanian general and nursing hospitals and influencing factors
A. Palekauskaite*, A. Berzanskute, R. Valienteiene (Vilnius, LT)

Background: Surveillance of antibiotic consumption and investigation of influencing it factors in hospitals is important tool of resistance control. In Lithuania only preliminary crude data on total consumption are available with limited information from hospitals. The main aim of our study was to determine antibiotic consumption and influencing factors in general and nursing hospitals.

Methods: Data on the consumption of antimicrobial agents for systemic use (ATC group J01) in 2007 and data on the influencing factors were collected by questionnaires sent to all general (66) and nursing (50) hospitals (response rate respectively – 71.2% and 82.7%). The ABCCalc from the WHO, version 3, was used to calculate the number of DDD/100 bed days of purchased antibiotics. Frequencies of existing influencing factors were used for comparison analysis. Differences between compared hospitals’ groups were accepted as statistically significant, when P < 0.05, counting Pearson’s χ² or Fisher’s exact test for proportions.

Results: There was huge variation of antibiotic consumption (ranging from 11.5 to 79.5 in general and from 0 to 26.6 DDD/100-bed days in nursing hospitals) and their structure between hospitals. The total antibiotic consumption was 40.6 DDD/100-bed days in general hospitals with three most used groups: penicillins (18.7 DDD/100-bed days), aminoglycosides (5.8) and cephalosporines (6.0). Antibiotic consumption in nursing hospitals was 9.7 DDD/100-bed days. The most used were penicillins (6.2 DDD/100-bed days), tetracyclines (1.5) and aminoglycosides (1.1). Only 25.5% of general and 2.1% of nursing hospitals have regulations on antibiotic prescribing. 44.7% of general and 16.7% of nursing hospitals have infection control specialists. None of the hospitals performs antibiotic consumption surveillance. In nursing hospitals higher antibiotic consumption was conditioned by lectures about antibiotic use organised by pharmaceutical companies. In general and nursing hospitals lower antibiotic consumption was conditioned by lectures given by universities.

Conclusions: The study revealed that antibiotic consumption in Lithuanian hospitals is not high. Big variations and expected misjudgment due to antibiotics purchased by patients themselves confirmed that the surveillance of antibiotic consumption should be stimulated in hospitals. Importance of some influencing factors indicated the need to revise regulations and audit procedures.
Results: The prevalence of FQ prescriptions among hospitalised patients were 9.7% in the 1st audit, 6.2% in the 2nd in 2007 and 4.2% in the 3rd in 2008. FQ were prescribed in patients treated for hematologic or solid tumours in 43%, 60% and 56.5% of cases in 2005, 2007 and 2008 respectively. FQ were prescribed in an empirical situation in 74% of cases in 2005, 50% in 2007 and 62.5% in 2008. The use of the intravenous (IV) route decreased from 45% in 2005 to 27% in 2007 and 34.8% in 2008 and was deemed not justified in 27% of IV prescriptions in 2005, 37.5% in 2007 and in 25% of cases in 2008. The unit dose of FQ was appropriate in 90% of prescriptions in the 3 audits and the frequencies of administration were appropriate in 92, 93 and 95% of prescriptions in 2005, 2007 and 2008 respectively.

Consumption of FQ in the hospital decreased from 145 defined daily dose per 1000 patient-days (DDD/1000 PD) in 2005 to 101 DDD/1000 PD and 95 DDD/1000 PD in 2007 and 2008 respectively (−34.5% between 2005 and 2008). Global institutional antibiotics consumption decreased by 18% between 2005 and 2008.

Conclusion: The intervention by the antimicrobial stewardship team contributed to the dramatic and sustained decrease in the consumption of FQ. The use of FQ in empirical treatments had also decreased but still remains high. The appropriateness of the route of administration (oral administration when possible) and of the unit dose could also be optimised. The results should be maintained and the quality of prescriptions reinforced.

**P762** Failure of a restriction list-based antibiotic policy to reduce the consumption of two antibiotics recently added to this list


Background and Objectives: According to an antibiotic restriction policy implemented in Hellenic hospitals since late 80s, all potent antibiotics (3rd and 4th generation cephalosporins, aztreonam, carbapenems, quinolones and glycopeptides and more recently linezolid, daptomycin and tigecycline) are included in a restriction list and can be dispensed by the hospital pharmacy only if the treating doctor fills in a specific form. However, ticarcillin–clavulanate (t/c) and piperacillin–tazobactam (p/t) were added to this list long after their introduction to the market and after a very successful course in Hellenic hospitals (at least for the latter). Our purpose was to study the impact of this kind of restriction policy on the consumption of t/c and p/t in our 300-bed hospital.

Methods: We retrospectively studied t/c and p/t consumption in our hospital by month from January 2007 to August 2008. We used data from the pharmacy computer. Antibiotic use was calculated in DDDs per 1000 patient days (ABC Calc 3.0 b). The values before and after restriction implementation (mid-June 2007) were studied with linear regression analysis (SPSS 11.5) for possible trend.

Results: The consumption of FQ in the hospital decreased from 145 defined daily dose per 1000 patient-days (DDD/1000 PD) in 2005 to 101 DDD/1000 PD and 95 DDD/1000 PD in 2007 and 2008 respectively (−34.5% between 2005 and 2008). Global institutional antibiotics consumption decreased by 18% between 2005 and 2008.

Conclusion: The intervention by the antimicrobial stewardship team contributed to the dramatic and sustained decrease in the consumption of FQ. The use of FQ in empirical treatments had also decreased but still remains high. The appropriateness of the route of administration (oral administration when possible) and of the unit dose could also be optimised. The results should be maintained and the quality of prescriptions reinforced.
Combinations mainly based on piperacillin-tazobactam, amoxicillin-clavulinate, cephalosporins, quinolones or glycopeptides never reached useful targets for quality of care improvement.

**Conclusion**: In this prospective study reflecting real life practice in ICU patients with nosocomial infections, the rate of appropriate or adequate empiric therapy was 64%. Empiric first-line use of meropenem allowed for the highest rates of appropriate or adequate therapy, irrespective of presence of risk factors for MDR involvement.

**Staphylococcus aureus bloodstream infection management indicators as quality indicators for hospital antibiotic stewardship: feasibility study by the ABS International Quality Indicators (ABS QI) team**


**Objectives**: The ABS QI team has developed a set of structural and process QIs as tools for evaluating hospital antibiotic stewardship programmes and auditing key treatment and prophylactic practices. Indicators related to the management of *Staphylococcus aureus* bloodstream infection (SAB) (echocardiography, intravascular (iv) catheter/device removal, effective therapy) were tested for feasibility, reliability and potential sensitivity to improvement in pilot hospitals.

**Methods**: Pilot hospitals participated in a review of all consecutive SAB cases admitted in 2007 and identified by microbiology laboratory databases. Three indicators (%age of patients [pts] with community-onset SAB who had echocardiography performed within 10 days after SAB onset [ECHO], %age of pts who had their iv catheter/device present at SAB onset removed within 10 days after SAB onset [CATH-EX], %age of pts with MS-SAB with a duration of iv betaalactam therapy of >10 days within the first 14 days after onset [BL-THER]) were assessed including data availability, reliability (tested on 25% of CRFs) and workload of QI measurement.

**Results**: A total of 494 SAB cases from 9 hospitals in 5 countries (3 in AT, 2 in BE, 2 in DE, 1 in CZ, 1 in SLO) were assessed. 60% of the pts were male (range between hospitals, 36–70%). The mean age was 62 yrs (58–70 yrs). 240 cases were community onset (49%, 19–96%), 320 had an iv device in place at SAB onset (65%, 28–77%), and 429 were MS-SAB (87%; 67–100%). 11% of the pts died within 14 days after SAB onset. Reliability was excellent (kappa >0.8) for the 3 QIs. The estimated median workload was 26 min per case assessment. Availability was 97%, 89%, and 87% for ECHO, CATH-EX, and BL-THER, respectively. In an intention-to-treat-analysis, QI values were: ECHO, 60% (9–75%); CATH-EX, 64% (30–78%); BL-THER, 60% (38–74%), respectively. As expected, per protocol QI values (excluding missing data and cases non-evaluable for other reasons) were slightly higher: ECHO, 62% (10–75%); CATH-EX, 72% (47–90%); BL-THER, 69% (44–82%), respectively. 51% of the echocardiographies and 83% of the device removals were performed within 3 days after onset.

**Conclusions**: The data demonstrate that these QIs can be reliably used across European acute care hospitals to retrospectively assess clinical compliance with recommended SAB management standards. Substantial inter-hospital variation in practice indicate that these indicators may be useful targets for quality of care improvement.

**Staphylococcus aureus bloodstream infection treatment and outcome of *Staphylococcus aureus* bacteraemia in nine European countries**


**Objective**: Inadequate empirical antibiotic therapy of *S. aureus* bacteraemia [SAB] has been associated with increased mortality and longer hospital stay. We aimed (1) to quantify inadequate empirical treatment in hospitalised patients with SAB in a representative sample of hospitals in nine West-European countries (Denmark, France, Germany, Italy, Netherlands, Spain, Sweden, Switzerland, UK); (2) to identify hospital-, patient- and microorganism-specific characteristics associated with inadequate treatment; and (3) to identify variables associated with 30-day mortality.

**Methods**: In a retrospective cohort study all adult patients with community- or hospital-acquired monobacterial SAB (mecillinam-susceptible [MSSA] or -resistant *S. aureus* [MRSA]), admitted to 60 randomly selected hospitals between 1 November and 31 December 2007, were eligible. Adequate antimicrobial therapy was defined as intravenous administration (with few exceptions depending on severity of illness and primary site of infection) of at least one antibiotic to which the isolate expressed in vitro susceptibility, that started (or was adequately adapted) within two days of the index blood culture or within one day if the patient had severe sepsis or septic shock. Quality of data was validated through checking of recorded data in 10% of randomly chosen case record forms in 31% of the participating hospitals.

**Results**: 334 SAB episodes (257 MSSA and 77 MRSA) were included. Ninety-five patients (28.4%) received inadequate empirical therapy (21.4% for MSSA and 51.9% for MRSA). Both length of stay (in days) before SAB and mecillinam resistance were associated with inadequate therapy with adjusted odds ratios of 1.01 (1.00 to 1.02) and 3.7 (2.1 to 6.4), respectively. Age (1.06 (1.03–1.10)), Charlson comorbidity score (2.1 (1.2–3.6)), severe sepsis or septic shock at time of SAB (2.7 (1.5–4.8)) and ICU stay at time of SAB (2.9 (1.5–5.6)), but not inadequate treatment (0.7 (0.4–1.3)) were associated with increased 30-day mortality. Based on the validity check 97% of eligible SABs had been included and 94% of the checked items were in accordance with inclusion criteria.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Alive (n=254)</th>
<th>Death (n=80)</th>
<th>OR (95%CI)</th>
<th>p-value</th>
<th>OR (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (vs female) (%)</td>
<td>170 (66.9)</td>
<td>54 (67.5)</td>
<td>1.03</td>
<td>0.67</td>
<td>1.03</td>
<td>0.80</td>
</tr>
<tr>
<td>Age (Adapted Charlson comorbidity score)</td>
<td>32 (12.6)</td>
<td>8 (10.0)</td>
<td>0.77</td>
<td>0.55</td>
<td>0.77</td>
<td>0.35</td>
</tr>
<tr>
<td>Secondary (vs primary) bacterium (%)</td>
<td>94 (37.0)</td>
<td>28 (35.0)</td>
<td>0.92</td>
<td>0.75</td>
<td>0.92</td>
<td>0.67</td>
</tr>
<tr>
<td>Length of stay before onset of SAB, median (QIR)</td>
<td>2.5 (0–10)</td>
<td>3.0 (0–5)</td>
<td>1.00</td>
<td>0.67</td>
<td>1.00</td>
<td>0.67</td>
</tr>
<tr>
<td>Severe sepsis or septic shock (vs sepsis) at onset of SAB (%)</td>
<td>77 (30.3)</td>
<td>45 (55.5)</td>
<td>&lt;0.001</td>
<td>2.68</td>
<td>&lt;0.001</td>
<td>2.68</td>
</tr>
<tr>
<td>ICI (vs non-ICI) at onset of SAB (%)</td>
<td>44 (17.5)</td>
<td>29 (35.5)</td>
<td>&lt;0.001</td>
<td>2.68</td>
<td>&lt;0.001</td>
<td>2.68</td>
</tr>
<tr>
<td>Inadequate (vs adequate) initial treatment (%)</td>
<td>73 (28.5)</td>
<td>20 (25.0)</td>
<td>1.36</td>
<td>0.46</td>
<td>1.36</td>
<td>0.46</td>
</tr>
<tr>
<td>MRSA (vs MSSA) (%)</td>
<td>57 (22.4)</td>
<td>20 (25.0)</td>
<td>1.15</td>
<td>0.64</td>
<td>1.15</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Table 1. Univariate and multivariate analysis for the outcome of 30-day all cause mortality
Clinical experience with daptomycin in Europe

A. Gonzalez-Ruiz, S. Daugaslé, A. Beiras-Fernandez, H. Lehmkuhl, P. Gargalianos-Kakolyris, Z. Daillana, A. Gallway, V.J. Gonzalez Ramallo, P. Dohmen, A. Skoutelis, R.A. Seaton, B. Almirante, G. Dognan, H.J. Thurston, R. Chaves (Dartford, UK; Basel, CH; Munich, Berlin, DE; Athens, Larissa, GR; Newcastle-upon-Tyne, UK; Madrid, ES; Glasgow, UK; Barcelona, ES; Bad Oeynhausen, DE)

Objective: To describe the clinical experience with daptomycin (DAP) in Europe since marketing authorisation in January 2006.

Methods: The European Cubin® Outcomes Registry and Experience (EU-CORE®SM) is a retrospective, non-interventional records review evaluating outcomes of patients (pts) receiving DAP. Investigators collected demographic, antibiotic, microbiological and clinical data from Jan 2006 to Sep 2008 from 118 institutions in nine European countries. Pts with multiple infection types were categorised by severity of infection (in order of decreasing severity: endocarditis, osteomyelitis, bacteremia, other [foreign body, septic arthritis, pyelonephritis/UTI, necrotising fasciitis], complicated skin and soft tissue infection [cSSTI], uncomplicated skin and soft tissue infection [uSSTI]). Outcomes were assessed by investigators using standard definitions.

Results: A total of 1127 pts were enrolled in EU-CORE®SM in the data reporting period. Of the pts in the safety population (n=1127), 64% were male, 46% were aged ≥65 years, 14% had CrCl <30 ml/min and 9% were on dialysis at the initiation of DAP therapy; 77% of pts were hospitalised prior to DAP therapy. The majority (89%) had significant underlying disease, including hypertension (30%), diabetes mellitus (26%) and chronic renal failure (13%). Primary infections included cSSTI (33%), bacteremia (22%), endocarditis (12%), vSSTI (11%), foreign body/prosthesis (8%) and osteomyelitis (6%). Clinical outcomes were success, defined as ‘cure plus improved’ (79%), failure (8%) and non-evaluable (13%). DAP was used as second-line therapy in 70% of pts, most frequently following therapy with glycopeptides (28%). DAP was given empirically in 53% of pts and methicillin-resistant Staphylococcus aureus infection was suspected in 29% of pts. Cultures were obtained in 91% of pts: the most frequently isolated pathogen was S. aureus of which 52% had confirmed methicillin resistance. The initial dose of DAP was 6 mg/kg in most pts (47%), whereas 32% of pts received 4 mg/kg and 20% received other doses. Outpatient DAP therapy was received by 14% of pts, whereas the majority were treated in hospital, where DAP was used with concomitant antibiotics in 67% of pts.

Conclusions: DAP was used to treat a range of infections, most frequently cSSTIs, with a large proportion of pts aged ≥65 years and with significant comorbidities. DAP was frequently used as second-line therapy, achieving an overall success rate of 79%.

Patient factors impacting the transition from inpatient to outpatient daptomycin treatment


Objectives: Outpatient parenteral antimicrobial therapy (OPAT) has increased dramatically over the past decade for multiple reasons. Recommendations for the clinical characteristics of patients appropriate for OPAT are largely based on expert opinion. Daptomycin has several characteristics that support OPAT (once daily administration, 30 minute infusion, well tolerated). This pilot study investigated patient factors associated with a transition to OPAT for daptomycin.

Methods: Patients (pts) were randomly selected from years 2005 and 2006 of Cubin Outcomes Registry and Experience (CORE) program data, which is an observational, multicentre, retrospective study that describes the clinical use of daptomycin. This pilot study examined the differences between those remaining as inpatients (IP) and those that began therapy as outpatients or transitioned from IP to OPAT (OP). Additional clinical data was collected for IP at the time daptomycin was initiated and for OP at the time OPAT was initiated or considered.

Results: Sixty endocarditis, bacteremia and skin and soft-tissue infection pts (20 IP, 40 OP) were randomly selected. The infection types by IP and OP, respectively, were: endocarditis (5; 25%); 5; 13%), bacteremia (9; 45%; 18; 45%), and skin (6; 30%; 17; 43%). One difference was found in underlying diseases, the IP group had a higher rate of cerebrovascular disease (15% vs 0%; P=0.03). The factors present in higher rates of IP pts were: abnormal respiratory status (45% vs 10%; P=0.02); abnormal chest x-ray (55% vs 15%; P=0.005); and a white blood cell (WBC) count >10,000/mm³ (75% vs 13%, P<0.001). Additionally, the IP group had a significantly higher median SAP (simplified acute physiology) score; 26 vs 16, P=0.001. The primary contributing to the higher SAP score in IP pts were: age, systolic blood pressure, serum urea nitrogen, and type of admission. Thirty-seven of the 40 (93%) OP pts completed their daptomycin therapy in the OPAT setting. Three pts who transitioned to OPAT subsequently were readmitted to receive daptomycin in IP; however, all 3 required inpatient treatment for a noninfectious underlying disease.

Conclusions: Of the factors investigated, abnormal respiratory status and elevated WBC were associated with pts remaining in the IP setting to receive daptomycin. A high percentage of pts who began or transitioned to OPAT daptomycin therapy were able to complete their daptomycin therapy in that setting.

Outpatient parenteral teicoplanin treatment in staphylococcal prosthesis infections

S. Sapmaz-Karabag, V. Avcan-Oguz*, N. Yapar (Izmir, TR)

Objectives: Isolation of the methicillin-resistant staphylococci from orthopedic joint prosthesis infections are on increase. Patients usually require intravenous antibiotic therapy and are treated while being hospitalised for long terms. In this study we aimed to investigate the results of the outpatient parenteral teicoplanin treatment for shortening the length of stay.

Methods: Fourteen patients between the time interval January 2006 and December 2007 prospectively and 18 patients between January and December 2008 prospectively were included in the study. The inclusion criterion was isolation of more than one staphylococcus on the patients’ microbiological culture taken during the prosthesis removal operation. Teicoplanin treatment (1-800 mg/day) was given to all cases. Inflammation parameters in the operation area and ESR, WBC, platelet counts, CRP values were followed up weekly.

Results: Of the patients, 20 were female (62.5%), 12 male (37.5%) and mean age was 65.72±10.87 (43–83). Twenty of the prosthesis (62.5%) were located in knee, 10 (31.3%) in hip and 2 (6.3%) in shoulder. Methicillin resistant coagulase negative staphylococcus (75%) was the most common strain with 24 patients and the second was methicillin resistant Staphylococcus aureus (15.6%) with 5 patients. Methicillin susceptible coagulase negative staphylococci were diagnosed in 2 (6.3%) patients. Only one patient was diagnosed to have Methicillin susceptible S. aureus (3.1%). No one had thrombocytopenia during the follow-up. Further radiological investigations were followed by surgical debridement in 8 cases that did not have a satisfying CRP decrease. Twenty three patients (71.9%) both clinically and laboratory responded the treatment, and new joint prostheses were placed. Five patients (15.6%) underwent surgical debridement. In 3 patients (9.4%) the treatment agent (teicoplanin) was changed and surgical debridement was performed. In one patient (3.1%) the treatment agent was changed because of the isolation a pathogen other than staphylococci. Performing surgical debridement and teicoplanin treatment succeeded in 87.5% of the cases, without performing surgical debridement the percentage of the treatment success rate was 71.9%.

Conclusion: As we did not observe any side effects and the success of treatment is high; the outpatient parenteral teicoplanin treatment can be an appropriate choice for the joint prosthesis infections.
Impact of nasal methicillin-resistant Staphylococcus aureus surveillance culture results on subsequent antibiotic prescribing patterns

J. Ruhe*, B. Kreisworth, D. Perlman, D. Mildcan, B. Koll (New York, Newark, US)

Objectives: Few data exist on whether the results of routine nasal MRSA surveillance culture (SC) may influence subsequent physician antibiotic prescribing patterns, especially with regard to the potential overuse of vancomycin.

Methods: Medical records were reviewed on all hospitalised patients (pts) with positive routine nasal MRSA SC between November 2007 and February 2008 (=cases). Zero time (ZT) was defined as the day the first nasal SC was performed. Data on antimicrobial agents administered between 3 days and 12 weeks after ZT were recorded. Pts with negative nasal staphylococcal cultures (=controls) were matched in a 1:1 ratio to case pts according to age, hospital ward at ZT, and length of hospital stay after ZT. Pts with active MRSA infections around ZT were excluded. MRSA isolates were characterised by multilocus sequence typing (MLST) and spa typing.

Results: Cases (n = 115) and controls (n = 115) did not differ significantly with regard to a variety of variables including sex, number of days in the intensive care unit, Charlson comorbidity score, and subsequent therapy with β-lactams or fluoroquinolones (P > 0.05 on bivariate analysis).

However, cases were more likely to develop MRSA complications within the observation period (8% versus 2%; P = 0.03). The mean duration of subsequent vancomycin exposure was 2.4 days (95% CI, 1.4−3.5) among cases and 0.7 days (95% CI, 0.4−1.1) among controls (P = 0.03). On multivariate analysis, a positive MRSA SC remained an independent predictor of subsequent vancomycin exposure (P = 0.037). 39 (45%) of 86 tested isolates belonged to clones that cause community-associated MRSA infections (MLST type CC8 and spa types 1or 7, respectively).

Conclusion: Pts identified as MRSA carriers were more likely to receive vancomycin within the subsequent 12 weeks than non-carriers, independent of other clinical characteristics such as infectious complications.

Cost-effectiveness of daptomycin in hospitalised patients with cSSTI caused by Gram-positive organisms


Objectives: Mortality associated with complicated skin and soft tissue infections (cSSTIs) due to MRSA infections has been increasing in the UK creating significant economic and humanistic burden. Daptomycin is a new, cyclic lipopeptide antibiotic for the treatment of cSSTI caused by Gram positive bacteria. Two randomised controlled trial showed similar overall clinical success rates for daptomycin vs. SSP or vancomycin (83.4% vs. 84.2%). However, 63% of patients successfully treated with iv daptomycin, required only 4−7 days of therapy, compared with 33% of comparator-treated patients (P < 0.0001). The aim of this study was to assess the cost-effectiveness and budget impact of daptomycin compared to vancomycin in hospitalised cSSTI patients with suspected MRSA infections.

Methods: A cost-minimisation model was developed from the perspective of the UK NHS (Figure 1), as due to the non-inferiority, similar efficacy was assumed between comparators. Only direct medical costs were considered. The outcomes assessed were total healthcare costs of treatment, including inpatient, laboratory tests, outpatient and drug costs. Resource use was collected based on information from a physician survey. Unit costs were extracted from publicly available databases. Probabilities and incidence of cSSTI were from a systematic literature review.

The time horizon was from hospital admission until resolution (i.e., less than a year), so no discounting was required. Uncertainty was explored in one-way sensitivity analyses and presented using a tornado diagram.

Results: Daily drug costs were £62.00 and £32.22 for daptomycin and vancomycin respectively. This cost differential was offset by the lower weekly monitoring costs (£39.42 vs. £114.07) and shorter hospitalisation for daptomycin. Total healthcare costs per patient were £3,756 and £3,841 respectively, resulting in £85 saving per person with daptomycin treatment. The main cost driver was hospitalisation, which was responsible for 84−85% of the total costs, while drug costs amounted to 9−12%. The introduction of daptomycin was estimated to save £110,491 in the first and £244,064 in the fifth year after the introduction of daptomycin as empiric therapy. The results were most sensitive to length of treatment with vancomycin, the number of days until first assessment of treatment failure and success rate with MRSA-induced infection.

Conclusion: Treatment of cSSTI with daptomycin is at least as effective as and less costly than vancomycin.

Utility of unique procalcitonin dosage in patients with acute exacerbation of chronic obstructive pulmonary disease in emergency wards

L. Blaïron, M.A. de Villenfagne, H. Dahma, P. Mols, A. Dediste*, O. Vandenberg (Brussels, BE)

Objectives: Some authors showed that procalcitonin (PCT) was able to distinguish patients with a bacterial aetiology of acute exacerbation (AE) of chronic obstructive pulmonary disease (COPD), resulting in a significant decrease in inappropriate antibiotics use. We tried to evaluate the ability of PCT to work in emergency conditions in this indication, and we compared the effect of a PCT-guided treatment vs the clinician’s intention to treat on the adequacy of the treatment.

Methods: We enrolled consecutive patients with AE-COPD in a six-month prospective, double-blind observational study. Every patient underwent a chest radiography and a supplemental blood puncture for differed dosage of PCT. PCT serum levels were assessed in batch with Vidas® B.R.A.H.M.S PCT (bioMérieux). Bacterial aetiology was suspected in the presence of suggestive clinical symptoms and either significant positive bacterial culture of sputum, or a positive atypical bacterial serology.
Results: Among 54 enrolled patients (median age 69.4; 95% CI 64.5–73.3), 17 had a bacterial infection (BI), 26 an atypical-bacterial infection (AI), 4 a proven viral infection (VI), 3 an isolated radiological focus (IRF), and 15 were considered as non-infected (NI). Some patients had mixed infections (BI+AI, or AI+VI). Median PCT serum levels (95% CI) were 0.05 ng/mL (0.05–0.53) in BI, 0.065 ng/mL (0.05–0.13) in AI, and 0.05 (0.05–0.10) in NI. In patients with isolated VI or IRF, median PCT values were both 0.05 ng/mL (CI non-applicable). Differences in PCT values among all these patients groups, and between infected vs non infected were both non significant (P=0.39 and P=0.21 respectively). Sensitivity and specificity of PCT were 46.2% and 73.3%. Antibiotic use should have been 8 on 39 infected patients in a simulation model of a PCT-guided treatment, and was 37/39 in standard conditions (OR=15.2; 95% CI 5.04–46.0; P<0.0005).

Conclusions: Bacterial aetiology of AE-COPD is difficult to prove in absence of evidence-based guidelines. Although PCT had been shown by some authors to reduce the antibiotics use, our experience showed that PCT could not be used safely in an emergency setting following the previously published recommendations in order to decide which AE-COPD patient should receive antibiotics.

**P772 Surveillance of antimicrobial use in Belgian hospitals**

S. Vaerenberg*, E. Hendrickx, B. Catty (Brussels, BE)

Objectives: To develop an easy to use and standardised method to monitor antimicrobial use in Belgian acute and long term (minimum 150 beds) care facilities, as part of a national programme to foster prudent antimicrobial use. This will allow individual hospitals to compare their own use with the national mean (benchmarking) and to analyse trends over time.

Methods: In Belgium, all antimicrobials for systemic use are reimbursed by the National Institute for Health Insurance and Disability and are identified by an unique Tarrification Unit Code (TUC). An electronic list of TUCs to monitor was defined and a secured web-based data upload module was created. Hospitals extract past calendar year data from their pharmacy database and upload them together with denominator data (bed days, admissions). Data are stored in a national database and the web module gives an immediate feedback of the numbers of DDD (Defined Daily Dose) used per 1000 bed days for each ATC code (Anatomical Therapeutical Classification, WHO, version 2008). The following drugs are monitored: antimicrobials for gastro-intestinal use (ATC-group A07A), antibiotics, antifungals and anticytotoxic for systemic use (D01BA, J01, J02&P01AB), and tuberculosis (J04A).

<table>
<thead>
<tr>
<th>Class</th>
<th>ATC</th>
<th>2006 (N=24)</th>
<th>2007 (N=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>range</td>
<td>mean</td>
</tr>
<tr>
<td>Beta-lactam antibiotics, penicillins</td>
<td>301C</td>
<td>235</td>
<td>168–375</td>
</tr>
<tr>
<td>Other beta-lactam antibiotics</td>
<td>301D</td>
<td>111</td>
<td>48–175</td>
</tr>
<tr>
<td>Quinolone antibiotics</td>
<td>301X</td>
<td>65</td>
<td>8–192</td>
</tr>
<tr>
<td>Other antibiotics*</td>
<td>301X</td>
<td>36</td>
<td>13–61</td>
</tr>
<tr>
<td>Antimycotics for systemic use</td>
<td>302A</td>
<td>26</td>
<td>0–72</td>
</tr>
<tr>
<td>Macrolides, lincosamides and streptogramins</td>
<td>301F</td>
<td>24</td>
<td>9–38</td>
</tr>
<tr>
<td>Aminoglycoside antibiotics</td>
<td>301G</td>
<td>15</td>
<td>3–38</td>
</tr>
<tr>
<td>Drugs for treatment of tuberculosis</td>
<td>304A</td>
<td>12</td>
<td>0–12³</td>
</tr>
<tr>
<td>Sulphonamides and trimethoprim</td>
<td>301E</td>
<td>9</td>
<td>1–22</td>
</tr>
<tr>
<td>Intravenous antibiotics</td>
<td>307A</td>
<td>4</td>
<td>0–21</td>
</tr>
<tr>
<td>Agents against anaerobes and other procarbolic disease</td>
<td>301A</td>
<td>3</td>
<td>0–7</td>
</tr>
<tr>
<td>Terfenadines</td>
<td>30A5</td>
<td>3</td>
<td>1–13</td>
</tr>
<tr>
<td>Antihistamines for systemic use</td>
<td>30HB</td>
<td>1</td>
<td>0–6</td>
</tr>
<tr>
<td>Amphotericin</td>
<td>301B</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>545</td>
<td>344–427</td>
<td>583</td>
</tr>
</tbody>
</table>

*Aminoglycoside antibiotics, polypeptide, sterol antibiotics, macrolide derivatives, trimethoprim derivatives and other.

**Results:** A total of 24 and 46 of the intended 37 and 61 hospitals participated in 2007 and 2008 (data for 2006 and 2007). The mean antimicrobial use was 545 (min 344, max 827) DDD/1000 bed days in 2006 and 583 (min 225, max 929) DDD/1000 bed days in 2007 (Table 1). Nineteen hospitals participated both in 2006 and 2007 and for these a small increase in antimicrobial use was seen. Especially beta-lactam antibacterials (301C) and anticytotoxic (302A) contributed to this effect. For 2007, the most frequently used molecules were ‘amoxicillin and enzyme inhibitor’, cefazolin and ciprofloxacin (70, 31 and 30 DDD/1000 bed days, respectively).

Conclusion: The web module allowed to collect standardised data from different hospitals. The participation rates (65% in 2007 and 75% in 2008) demonstrate that this surveillance system is feasible for Belgian hospitals after a relatively short time of notification. Antimicrobial use could be compared between hospitals and between years. Further data collection will also enable analysis of trends and incorporation of more indicators will further increase the usefulness of this web module as a tool to guide antibiotic policy in hospitals.

**P774 Improving patient safety in Europe. Standards and performance indicators in healthcare-associated infection and antimicrobial stewardship**

B. Cookson*, D. Mackenzie, A.P. Coutinho, A. Gilsdorf, J. Russell, J. Fabry (London, UK; Copenhagen, DK; Lyon, FR)

Objectives: To achieve a consensus on Standards and Performance Indicators (SPI) to assess Healthcare Associated Infection (HCAI) prevention and control programmes and antimicrobial stewardship (AMS) in European countries and to try and produce a reduced set of SPI (RSSPI).

Methods: We used several sources to draft HCAI/AMS SPI and used a novel Likert spreadsheet approach from an EU project (HARMONY) to assess consensus and analyse responses from 29 of 33 European countries approached. Since the 2008 ECCMID, these have been finalised interactively. Several countries and ECDC wanted to develop an RSSPI and we used the same approach to do this.

Results: National and local SPI had been developed in five groups with 144 statements. Despite the high consensus achieved in the first round there were many comments received and the advisory group agreed 64 modifications to the SPI (and recommended practices) to further increase their acceptance. The second round of interactions and consensus conference in May 2008 showed we had effectively addressed the issues. Four ways of publishing surveillance data were proposed to circumvent the differences in attitudes in several countries. Staffing and isolation requirements need further consensus work in Europe. 13 international and 13 national RSSPI were all felt to be important by the 24 responding countries. For example “Recording training at staff induction”, the lowest scored national indicator, had an average priority of 7.52 (out of 13), with three responses placing it in the top three and six in the top five. The modal prioritisation for eight of the national indicators and six of the international indicators was of the highest possible. It was thought to be important to develop and agree a validation process for SPI/RSSPI and that there should be a core group of SPI which could be repeated regularly, with others that could be reviewed over time and perhaps even changed?

Conclusions: We believe this to be the most rigorous consensus ever attempted in the field and it is reassuring to see the level of agreement realised. The methods used have again shown their ability to rapidly establish a consensus in a multi-faceted landscape. It is anticipated that others will find their use as productive. We have also developed a RSSPI which will be explored further with ECDC in the coming years.

**P775 Linezolid for patients with neutropenia: can bacteriostatic agents be used in this patient population?**

P. Rafailidis*, V. Kouranos, C. Christodoulou, M. Falagas (Athens, GR)

Objective: A long held doctrine is that bacterioidal antibiotics are required for infections in neutropenic patients. We sought to review the available data concerning the clinical use of linezolid, a bacteriostatic antibiotic, in the treatment of infections in neutropenic patients.

Methods: We evaluated the available published evidence (PubMed) regarding the role of linezolid, a bacteriostatic antibiotic, in neutropenic
patients with *Staphylococcus aureus*, *Enterococcus faecalis*, or *Enterococcus faecium* infection.

**Results:** We retrieved three non-comparative studies, two comparative studies [one of them is a double blind randomised controlled trial (RCT)], two retrospective studies and eight case reports that focused on the use of linezolid for Gram-positive bacterial infections in neutropenic patients. Linezolid was administered to 438 neutropenic patients, mainly on a compassionate-use basis, as other antibiotics failed to cure the infection or were associated with significant adverse events. In total, 62 out of 438 (14.1%) neutropenic patients that received linezolid died during therapy. In the only RCT that compared linezolid to vancomycin in the treatment of Gram-positive infections in neutropenic patients, mortality was 5.6% versus 7.6%, respectively (p = 0.4).

**Conclusion:** The available evidence suggests that linezolid is successful in a significant proportion of neutropenic patients with infection, despite the fact that it is a bacteriostatic agent. Such data seem to justify further studies regarding the role of bacteriostatic agents, including linezolid, in this patient population.

**P775 What determines regional differences in outpatient antimicrobial consumption in the Russian Federation?**

A. Fokin*, S. Ratchina, S. Kozlov, I. Guadko, A. Ishmakhametov, M. Denisova (Smolensk, Moscow, RU)

**Objectives:** We aimed our study to compare outpatient antimicrobial consumption (AC) in different regions of Russian Federation (RF) and investigate socio-economic determinants of AC.

**Methods:** AC data in ATC class J01 were obtained by RGC in 2004–2006 during pharmacy audit in 11 regions of RF and expressed as number of DDD per 1000 inhabitants per day (DDI) (ATC-DDI Index, 2007). Demography and socioeconomic indexes: population, population density (v2), urban sector weight, men/women ratio, age structure, natural increase and marriage (v9) rates, economically active population weight, economic activity level, unemployment rate, average per capita population monetary income (v13), average monthly nominal accrued salary for working in economics (v14), average rate of monthly pension assigned (v15), pensioners quantity, population with monetary incomes lower living-wage weight (v17), medical institutions quantity, hospital beds quantity (v19), hospital beds quantity/1000 inhabitants (inh), number of inh/hospital bed, out-patients’ clinics visits/day (v22), out-patients’ clinics visits/day/1000 inh, physicians quantity (v24), physicians quantity/1000 inh, number of inh/physician, nurses quantity, nurses quantity/1000 inh, number of inh/nurse, morbidity, some infectious and parasitic diseases morbidity, retaillment turnover, retaillment turnover per capita, gross regional product per capita were taken from RF Federal agency of state statistics report. To assess correlation of variables multiple regression analysis was performed using SAS (program package SAS Institute, USA, version 8.02 for Windows XP).

**Results:** AC in 11 regions of RF in 2004–2006 is presented in Table.

<table>
<thead>
<tr>
<th>Region</th>
<th>AC (DDD)</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voronezhskaya oblast</td>
<td>11.08</td>
<td>12.9</td>
<td>12.23</td>
<td></td>
</tr>
<tr>
<td>Krasnodarskiy kray</td>
<td>8.62</td>
<td>7.08</td>
<td>6.08</td>
<td></td>
</tr>
<tr>
<td>Krasnoyarskiy kray</td>
<td>9.16</td>
<td>9.59</td>
<td>9.62</td>
<td></td>
</tr>
<tr>
<td>Nizhgorodskaya oblast</td>
<td>8.09</td>
<td>8.59</td>
<td>8.37</td>
<td></td>
</tr>
<tr>
<td>Novosibirskaya oblast</td>
<td>10.04</td>
<td>12.24</td>
<td>12.43</td>
<td></td>
</tr>
<tr>
<td>Omskaya oblast</td>
<td>6.40</td>
<td>7.29</td>
<td>7.26</td>
<td></td>
</tr>
<tr>
<td>Bashkortostan</td>
<td>9.74</td>
<td>11.06</td>
<td>11.88</td>
<td></td>
</tr>
<tr>
<td>Tatarstan</td>
<td>8.92</td>
<td>9.92</td>
<td>10.45</td>
<td></td>
</tr>
<tr>
<td>Rostovskaya oblast</td>
<td>4.94</td>
<td>7.12</td>
<td>6.93</td>
<td></td>
</tr>
<tr>
<td>Samarskaya oblast</td>
<td>8.55</td>
<td>7.76</td>
<td>8.38</td>
<td></td>
</tr>
<tr>
<td>Sverdlovskaya oblast</td>
<td>7.94</td>
<td>7.38</td>
<td>7.19</td>
<td></td>
</tr>
</tbody>
</table>

After multiple regression analysis carrying out we defined dependence model: DID = 9.3905 – 0.15×v2 – 3.8311×v9 – 9.3132×ln(v13) – 11.1692×ln(v14) + 0.0154×v15 – 0.3590×v17 – 0.6394×v19 – 0.1126×v22 + 48.0576×ln(v24) (R2 = 0.849, adjusted R2 = 0.789).

**Conclusion:** Regions differed significantly in level of AC in study period. Some variables – v15 and v24 exerted positive influence on AC, some – v2, v9, v13, v14, v17, v19 and v22 – negative one.

**P776 A survey on current antibiotic prescribing attitude of primary care physicians in Greece**


**Objectives:** Greece is the leading country in antibiotic consumption in Europe in the outpatient setting. A public survey conducted in Greece a few years ago indicated that more than 75% of antibiotics used in the community were prescribed by primary care physicians (PCPs). A survey was performed to investigate the prescribing attitude in clinical practice among PCPs.

**Methods:** An anonymous questionnaire was mailed or distributed during the annual meetings of paediatrcians, internists, pneumonologists and general practitioners (GPs), addressing indications of antibiotic use, antibiotic choices and factors affecting prescribing.

**Results:** 1416 questionnaires were finally collected at a national scale during winter 2007–2008 (from 230 paediatricians, 178 pneumonologists, 232 GPs and 776 internists). GPs were mostly working for the state (78%), practicing in rural areas (80%), while the rest were mostly private practice physicians working in urban areas (65–85%). The commonest indication for antibiotic use was respiratory tract infections (70–90%), including primarily exudative tonsillitis, COPD exacerbations and pneumonia in adults, and prolonged nasal purulent discharge along with otitis media in children. Antibiotics most commonly prescribed were macrolides, second generation cephalosporins and amoxicillin or amox/clav. Antibiotic choices followed guidelines for acute cystitis, tonsillitis and otitis media, but were inappropriate in pneumonia (40%) and COPD (30%) and overused in acute diarrhoea (33–40%), patients with indwelling urinary cathetars (40–60%) and patients with viral symptoms (50%). Eighty five percent admitted prescribing retrospectively antibiotics to their patients which had been already self-administered and >50% included pharmaceutical reps in their sources of new information.

**Conclusions:** Campaigns for the prudent use of antibiotics should aim at PCPs. New information, education and guidance should be continuously offered.

**P777 Public campaigns to improve outpatient antibiotic use in high-income countries**

B. Huttner*, S. Harbarth on behalf of the CHAMP Consortium

**Objective:** Public campaigns have attempted to educate the public regarding prudent outpatient antibiotic use. We reviewed characteristics and outcomes of these campaigns as part of an international collaborative project.

**Methods:** Through Medline, internet searches and contact with expert informants, we identified public campaigns aimed at improving antibiotic use conducted on a national or regional level in high-income countries between 1990 and 2007. Campaign managers were contacted to obtain unpublished information. Randomised trials and campaigns carried out on a community level were excluded. Analyses were performed using a mixed approach (quantitative and qualitative methods).

**Results:** We retrieved information on 16 national campaigns and 6 regional campaigns (16 in Europe, 3 in North America, 2 in Oceania and 1 in Israel). All but 4 campaigns were conducted over more than 1 year (range, 1–13 y) and 12 campaigns were still ongoing in 2007. Most campaigns (n = 17) were organised by health authorities and publicly funded. Two national campaigns were funded by the
pharmaceutical industry. All campaigns focused on upper respiratory tract infections and used similar key messages. All but one campaign targeted physicians and the public in parallel, with an emphasis on parents of young children (n = 17). Interventions were multifaceted and varied in intensity. Distribution of information material was the most common intervention (n = 22). Twelve campaigns used television and 2 campaigns used intensive academic detailing for physicians. Nine campaigns observed a reduction in antibiotic prescriptions and 2 campaigns in self-reported antibiotic use. The impact on antimicrobial resistance was difficult to evaluate because of poor data availability and the concomitant introduction of the pneumococcal conjugate vaccine in several countries. Potential adverse outcomes and sustained effects have not been evaluated systematically.

Conclusions: Antibiotic campaigns are widely used and some have resulted in a reduction in antibiotic use, although a clear cause-effect relationship is difficult to establish. The lack of detailed evaluation, the multifaceted approach and the differences in healthcare systems make identifying the most effective interventions a challenge. Although the impact on antibiotic resistance is difficult to assess at the current moment, policy makers and epidemiologists can use our findings to develop initiatives suited to different country settings.

**P778** Linezolid versus vancomycin health and economic outcomes: a retrospective database study of 11,018 infection-related hospitalisation treatment episodes


Objectives: Vancomycin has been the standard drug for infections with specific aetiologies, including methicillin-resistant *Staphylococcus aureus*, but there is increasing evidence from large comparator controlled trials that linezolid may have clinical advantages over vancomycin. The objectives of this study were to examine the likelihood of repeat hospitalisation and to compare the post-index hospitalisation costs of a cohort of discharged patients who were treated for their infection with either linezolid or vancomycin.

Methods: Data came from 3 separate administrative claims databases. Adult non-Medicare patients were required to be on either vancomycin or linezolid and treated for an identifiable infection. The index date reflects the date that the index drug was dispensed. Patients were excluded if they were not continuously enrolled for at least 6 months following index. Analyses were adjusted for comorbidities, age, sex and geography, as well as the index infection. Adjusted odds ratios were calculated to determine the relative impact of linezolid versus vancomycin on the likelihood of a repeat hospitalisation following initiation of treatment.

Results: The proportion of patients with re-hospitalisation following their index date was 26.1% in the linezolid group (759/2907) and 33.4% (2706/8111) in the vancomycin group. The relative odds of a post-index hospitalisation in the adjusted logistic regressions ranged from 0.830 to 0.839. Furthermore, the post-index costs were statistically lower for patients on linezolid compared to those on vancomycin.

Conclusion: The unadjusted likelihood of a post-index re-hospitalisation was 7% lower in absolute terms or 22% lower in relative terms for those on linezolid versus vancomycin. The adjusted rates, controlling for the index infection, comorbidities, age, sex, and geography found relative reductions in the range of 16% to 17%. Linezolid was also associated with lower post index adjusted and unadjusted costs.

**P779** Molecular bacteriology – part 1

**P779** Testing for *Clostridium difficile* infection

J. Swinndles*, N. Brenwald, N. Reading, B. Oppenheim (Birmingham, UK)

Objectives: Recently concerns have been raised regarding the reliability of many of the methods that are currently utilised by laboratories for the diagnosis of *Clostridium difficile* infection (CDI). In addition, several new alternatives have become available, including the development of polymerase chain reaction (PCR) tests. We sought to compare the performance of four rapid tests against the gold standards of cytotoxicity assay and culture.

Methods: To date 71 consecutive diarrhoeal specimens taken from inpatients aged over 65 have been analyzed using the following rapid tests: VIDAS® *Clostridium difficile* A&B (bioMerieux) enzyme immunoassay (VIDAS EIA), XpertTM *C. difficile* PCR (Cepheid), Gene OhmTM PCR (BD Diagnostics) and C. DIFF QUIK CHECK COMPLETE™ (TechLab). The latter of these tests independently detects both glutamate dehydrogenase antigen (GDH) and *C. difficile* toxin (CTD). The stool samples were cultured for growth of *C. difficile* according to standard methods. Cytotoxicity assay was undertaken using Monkey African Green Kidney ‘Vero’ cells on each stool sample and any positive culture isolates.

Results: *C. difficile* was cultured from 14% of specimens. The C. DIFF QUIK CHECK COMPLETE™ GDH component identified all of these, with a sensitivity of 100% and specificity of 98.4%. The stool cytotoxicity assay was positive for 10% of specimens, giving calculated sensitivities of 100% for each of the PCR tests compared to 71.4% for both the C. DIFF QUIK CHECK COMPLETE™ CDT component and VIDAS EIA (see table).

<table>
<thead>
<tr>
<th>Test under evaluation</th>
<th>Gold standard used for comparison</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. DIFF QUIK CHECK COMPLETE™</td>
<td>GDH antibody</td>
<td>100</td>
<td>99.4</td>
<td>99.9</td>
<td>100</td>
</tr>
<tr>
<td>C. difficile culture</td>
<td>100</td>
<td>99.4</td>
<td>99.9</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>VIDAS® <em>Clostridium difficile</em> A&amp;B</td>
<td>Cell cytotoxicity assay</td>
<td>71.4</td>
<td>100</td>
<td>100</td>
<td>97.0</td>
</tr>
<tr>
<td>Xpert™ <em>C. difficile</em> PCR</td>
<td>Cell cytotoxicity assay</td>
<td>100</td>
<td>96.9</td>
<td>77.8</td>
<td>100</td>
</tr>
<tr>
<td>Gene Ohm™ PCR</td>
<td>Cell cytotoxicity assay</td>
<td>100</td>
<td>96.9</td>
<td>77.8</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value.

**P780** Determining hypervirulent markers for *Clostridium difficile* by array comparative genomic hybridisation

G. Marsden*, J. Davis, V. Wright, J. Hinds, E. Kuijper, N. Minton (Nottingham, London, UK; Leiden, NL)

Objectives: The aim of the study was to use a comparative genomic approach for the identification of hypervirulent markers in *Clostridium difficile*. The objective was to use a comparative genomic approach for the identification of hypervirulent markers in *Clostridium difficile*.

Methods: A high density oligonucleotide microarray was designed to the *Clostridium difficile* 630 sequence and constructed by Oxford Gene Technology (UK). Extra probes were also designed to regions of divergence in the un-annotated sequences of two PCR ribotypes O27 strains, *C. difficile* R20291 (WT51, UK) and QCD-32 g58 (McGill University, Canada) when compared to *C. difficile* 630 and regions of interest, such as the agr operon. A set of 34 clinical strains comprised of the most commonly represented PCR ribotypes throughout Europe, including type O27 was hybridised to the microarray. These included 21 ribotype O27 strains originating from Europe, the USA and Canada, and 7 strains representing the subtypes of ribotype 001. Strains representing the new emerging ribotype 078 have also been hybridised to the microarray.

Analysis of the microarray data was performed using GeneSpring GX v7.3 (Agilent, USA).

Results: Microarray analysis revealed distinct regions of divergence amongst the strains analysed in comparison to *C. difficile* 630 across all
PCR ribotypes tested. Comparison of O27 strains from North America showed genetic differences or divergence from R20291 specific probes, yet probes designed specifically to the Canadian strain QCD-32 g58 appear to hybridise consistently to all European O27 strains.

**Conclusion:** Array comparative genomic hybridisation (aCGH) successfully identified regions of divergence that could be used as markers for O27 strains as well as markers for *Clostridium difficile* 630.

**P781** Toxin genes (tcdA, tcdB, cdtA and cdtB) of *Clostridium difficile* strains isolated from patients with *Clostridium difficile*-associated diarrhoea in Turkey

U. Deniz, N. Ulger Toprak*, B. Aksu, G. Soyletir (Istanbul, TR)

**Background:** *Clostridium difficile* is the causative agent of a spectrum of gastrointestinal syndromes in humans ranging from diarrhoea to severe life-threatening colitis. Pathogenesis primarily involves the action of two large clostridial cytotoxins, toxin A and toxin B, which are encoded by the tcdA and tcdB genes, respectively. Reports on toxin A variant strains (tcdA negative, tcdB positive), and the recent emergence of the epidemic binary toxin positive *C. difficile* strains in Europe, USA and Canada lead to important changes in epidemiology of *C. difficile*-associated diarrhoea (CDAD).

**Objective:** This study investigated the toxin genes of *C. difficile* strains isolated from hospitalised patients with antibiotic-associated diarrhoea (AAD).

**Methods:** In the period of September 2006-March 2008, the stool samples from 633 patients with nosocomial AAD at Marmara University Hospital were analyzed for *C. difficile* by culture, toxin A/B immunoenzymatic detection (Immunocard Toxins A&B, Meridian Diagnostics, Inc., Ohio, USA). In addition, culture filtrates of the isolates were also screened for the toxin A/B by immunoassay test. Genes for toxin A (tcdA), toxin B (tcdB), binary toxin (cdtA and cdtB) were determined by PCR.

**Results:** Fifty stool specimens yielded *C. difficile* on culture; while only 30 of these were positive by toxin immunoassay test. However, an additional 6 samples which were negative by direct toxin test were found to be toxin positive when assay was performed on culture filtrates of the *C. difficile* isolates, giving a sensitivity for direct toxin assay as 88%. The toxin A and toxin B genes were detected in all strains (n: 36) isolated from samples that were toxin positive either directly or from culture filtrates. There were neither variant strains (tcdA-negative and tcdB-positive) nor binary toxin gene positive isolates among tested bacteria.

**Conclusion:** Our findings form a database about toxin genes of *C. difficile* in hospitalised patients with AAD in Turkey, where molecular investigation of toxin-producing *C. difficile* strains has not been performed so far. Despite absence of isolates producing new toxin variants or binary toxin in this study, it seems to be important to monitor the isolates for the emergence of those strains which still cannot be detected by commercially available tests, in order to control and prevent the outbreaks.

**P782** Proof of antigenic diversity among the cytotoxins TcdB and use of novel monoclonal antibodies to diagnose *Clostridium difficile* ribotype strains


During recent years several outbreaks of *Clostridium difficile* infection have been related to single ribotypes like 017 and 027. Ideally it should become possible to detect endemic ribotypes with a quick bedside test. The aim of the current study was to prepare TcdB of different *C. difficile* strains and to use the polypeptides for immunisation and later induction of hybridoma cells that could be used to differentiate the most prominent *C. difficile* ribotypes mainly according to their variant TcdB molecules. TcdB was isolated from standard strain *C. difficile* VPI10463, of the ribotype 017 strain 1470 and of ribotype 027 strain LUNC4. The toxins were inactivated and used for immunisation of mice. Thus app 30 novel monoclonal antibodies were generated. Testing was done by ELISA, Western blotting and to mAbs that were identified that differentiate between different toxins. The monoclonal antibodies obtained could be classified into three major groups. The first group called pan-TcdB specific mAbs represents antibodies that recognize all TcdB molecules that we have in hand. A second group of antibodies (TcdB-027 specific) recognize TcdB of ribotype 027 but not of ribotype 001. The third group contains antibodies that react ribotype 001 TcdB but not TcdB-027 (TcdB-001 specific). Combinations of these antibodies were tested for their potential to diagnose 027 versus 017 or 001 ribotype infections.

Our experiments demonstrate that not every monoclonal reacts with all TcdB molecule of all different strains. Only a fraction of mAbs is pan-TcdB specific. Lack of pan-TcdB specificity might be an obstacles of some commercial tests with deficits in sensitivity. Further our results prove that monoclonal antibodies can be generated that differentiate between a ribotype 027 infection versus infections with other ribotypes. In collaboration with an accompanying company a prototype test was developed that allows the diagnosis of a ribotype 027 infection within less than 20 min.

Our data demonstrate that immunogenic testing will become an appropriate approach for quick diagnosis of the most abundant endemic *C. difficile* strains. Testing on the most abundant European ribotypes will be presented and the use of such testing for diagnosis of CDI will be discussed.

Acknowledgement: This work was support as part of the EACCAD FP6 European grant coordinated by Dr. Ed Kuijer (Leiden University Medical Centre)

**P783** Evaluation of a combined toxinA/B PCR for the detection of *Clostridium difficile* in a general hospital

L. Smeets*, I. Pollard, P. van Wunnik, E. van der Vorm, M. Kooistra-Smit, R. de Boer (Delft, Groningen, NL)

**Objective:** To evaluate a combined (duplex) real-time PCR for the detection of the *Clostridium difficile* toxin A and toxin B for the detection of *C. difficile* associated diarrhoea (CDAD) in a general hospital.

**Methods:** Stool samples for *C. difficile* were first tested with a cytotoxicity test on Human embryonic lung (HEL) cell culture (CTA) and if positive the faeces were cultured for *C. difficile*. All stool samples were stored at −80°C and screened retrospectively with PCR. DNA extraction was performed with the Xtractor Genie (Corbett Robotics) and PCR on both the tcdA and tcdB genes was performed on an ABI prism 7500 thermocycler. If the PCR was inhibited, extraction and PCR were repeated after 1:5 dilution of the sample in saline. If the three tests (CTA, culture, and PCR) yielded discrepant results, the combination of two independent results was considered to be the consensus gold standard. If only two independent results were available, the sample was withdrawn from the study.

**Results:** In total 143 samples were tested with CTA and PCR (Table 1). Of 15 samples that were positive in both the CTA and culture, 12 were also PCR positive. Of the 15 samples that were CTA positive but culture negative, none were PCR positive. These samples were thus regarded true negatives. Of the 113 samples that were CTA negative, 110 were also PCR negative and 3 PCR positive. One of these was also positive in subsequent culture from the frozen faeces and thus considered to be a true positive. This patient had an earlier faecal sample that was CTA positive, indicating that a toxigenic strain was present. The remaining two CTA negative, PCR positive samples were not available for culture. One of these patients had repeatedly negative CTA tests.

**Table 1. Results of CTA, PCR and (if available) culture of all 143 samples**

<table>
<thead>
<tr>
<th>CTA−</th>
<th>CTA+/culture−</th>
<th>CTA+/culture+</th>
</tr>
</thead>
<tbody>
<tr>
<td>110 negative, 3 positive†</td>
<td>3 negative, 12 positive</td>
<td>15 negative</td>
</tr>
</tbody>
</table>

*Culture not performed. †1/3 culture positive, 2/3 not available for further analysis.
Altogether, for 141 samples a consensus result could be obtained (Table 2). The sensitivity of the PCR was 81.3%, the specificity 100%. For CTA these figures are 93.8% and 88.0%, respectively.

Table 2. CTA and PCR results compared with the consensus gold standard

<table>
<thead>
<tr>
<th></th>
<th>Negatives (125)</th>
<th>Positives (16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTA</td>
<td>110 negative (specificity 88%)</td>
<td>15 positive (sensitivity 93.8%)</td>
</tr>
<tr>
<td>PCR</td>
<td>125 negative (specificity 100%)</td>
<td>13 positive (sensitivity 81.3%)</td>
</tr>
</tbody>
</table>

Conclusion: In comparison to the CTA test on Hel-cells, the combined tcdA/tcdB PCR for C. difficile has a higher specificity but lower sensitivity. Theoretically, the presence of tcdA/tcdB is no evidence for actual toxin production. In our 143 samples however, only two potentially contained non-toxigenic strains. The presence of the toxin genes (as detected by PCR) therefore appears to be a reliable indicator for C. difficile toxin production.

**P784** Rapid detection of Clostridium difficile in faeces by real-time PCR

K. Egli, S. Peter-Getzlaff, M. Altwegg* (Lucerne, Zurich, CH)

Objectives: Traditional methods for the detection of C. difficile consist of immunoassays (sometimes believed to lack sufficient sensitivity), culture on selective medium, and traditional “gold standard” tissue culture cytotoxicity methods, which are difficult to perform and require several days to yield results. Our study was aimed at determining whether a commercial and a home-brew real-time PCR are suitable for the rapid detection of C. difficile in faecal specimens.

Methods: As part of an ongoing study for determining the performance of culture, cell culture cytotoxicity and three immunological toxin tests, a subset of 133 unformed stool specimens from patients with suspected CDAD was also analyzed by the BD GeneOhm™ Cdiff Assay (BD-PCR) consisting of a rapid, glass bead-based DNA preparation without further purification followed by real-time amplification of part of the toxin B gene on a SmartCycler II. The same extract was simultaneously analyzed with a home-brew duplex PCR detecting both toxin A and B genes using the 5’ exonuclease format on a LightCycler 480 (BA-PCR). The performance of PCR was compared to the ‘gold standard’ (cell culture cytotoxicity, with culture as reference for the resolution of discrepant results). 131 specimens were also analyzed by commercial and home-brew PCR after extraction/purification of DNA from specimens with the easyMAG system.

Results: DNA preparation according to the BD GeneOhm™ Cdiff Assay (N=133) resulted in inhibition of 3 (2.3%) and 11 (8.3%) of specimens with BD- and BA-PCR, respectively. Sensitivity/specificity for not inhibited specimens was 95.7%/96.4% for BD-PCR, 95.1%/97.5% for BA-PCR, and 89.1%/98.8% for cell culture cytotoxicity. After easyMAG extraction (N=131), 2 (1.5%) and 1 (0.8%) of specimens showed inhibition in BD- and BA-PCR, respectively. Sensitivity/specificity for not inhibited specimens was 100%/97.5% for BD-PCR, 97.6%/96.3% for BA-PCR, and 89.8%/100% for cell culture cytotoxicity.

Conclusions: Both PCR assays are more sensitive than and almost as specific as cell culture cytotoxicity and thus provide simple and rapid stool tests that allow same-day identification of toxigenic C. difficile. DNA purification slightly increases the performance of the commercial assay and is a must for our home-brew PCR in order to avoid too many non valid results due to inhibition.

**P785** A two-step glutamate dehydrogenase antigen: real-time polymerase chain reaction assay for detection of toxigenic Clostridium difficile

S. Goldenberg*, P. Cliff, G. French (London, UK)

Objectives: Most diagnostic laboratories in the UK and Europe use one of several commercially available Enzyme Immunoassays (EIA) to diagnose Clostridium difficile infection (CDI). These assays detect toxins A/B but suffer from having a poor sensitivity compared to Cytotoxin assay (CTN) which is considered the gold standard). We evaluated a new two-step algorithm which utilises the common antigen Glutamate Dehydrogenase (GDH) as a screening step, followed by confirmation using real time PCR for detection of the toxin B gene (tcdB).

Methods: 500 consecutive diarrhoeal stool samples were tested using the two-step protocol and compared to testing by a commercially available EIA, according to manufacturers instructions. To confirm the true status of the sample, toxigene culture was also performed at the same time as the other tests.

Results: Of 500 specimens tested, 399 (79.8%) were negative for GDH and EIA. 80% of samples were GDH positive and went on to have RT-PCR performed. Among the 16 samples that were EIA positive, 13 were also GDH positive, RT-PCR positive, were isolated in culture and were shown to be producing toxin. Two samples were GDH positive but RT-PCR negative. C. difficile was cultured from one of these samples and was shown to produce toxin. Of the 484 samples that were EIA negative 85 (17%) were GDH positive. 23 of these samples were RT-PCR positive and organism was cultured from 21 of these. Toxin production was demonstrated in 20 samples that were cultured and were considered true positives.

Among the 62 samples that were GDH positive and RT-PCR negative, organism could not be cultured in 58. Organism was cultured from the remaining 4 samples, and only one of these demonstrated toxin production and was considered to be true positives. Toxin production could not be demonstrated in the other three samples and were considered to be non-toxigenic.

Comparing EIA to toxigenic culture sensitivity and specificity were found to be 40% and 99.6% respectively. (PPV=87.5% NPV=95.6%). Comparing the two-step algorithm to toxigenic culture demonstrated a sensitivity and specificity of 94.3% and 100% respectively (PPV=100% NPV=99.5%).

Discussion: The two-step algorithm was found to be more expensive than the EIA assay (an additional cost of £GBP 8000 based on 500 samples tested per year). However the two step algorithm enabled negative samples to be rapidly excluded and led to markedly fewer false negatives whilst causing minimal diagnostic delay.

**P786** Analysis of Clostridium difficile binary toxin gene expression

D.S Metcalf*, J.S. Weese (Guelph, CA)

Objective: The major Clostridium difficile toxins, TcdA and TcdB, are well characterised virulence factors, however many toxigenic strains also possess cdtAB genes encoding a binary toxin. The role of the binary toxin is currently poorly understood as is its regulation of expression. The objective of this research was to characterise the expression of cdtA, and the upstream putative regulator, cdtR. In addition, complete sequencing of the cdtAB locus is being performed to evaluate inter-strain variability and identify other potential regulators of binary toxin gene expression.

Methods: Eleven cdtAB positive C. difficile isolates from 9 different ribotypes were evaluated. RNA was isolated from exponential and stationary phases of growth, reverse-transcribed, and real-time PCR was used to compare expression of cdtA and cdtR, using rpoA as a reference gene. Testing was performed in triplicate and 24 primer pairs were designed and used to sequence the complete ~6.5 kb binary toxin locus of all 11 strains.

Results: There was a significant difference in expression of both cdtA and cdtR between exponential and stationary phases (P < 0.05), with significantly higher expression in stationary phase. There was not a statistically significant correlation between cdtA and cdtR, however that may be a limitation of statistical power. There was no association between the level of expression of either gene and ribotype or toxigenotype (P > 0.05). Although the CDT locus is mostly conserved, several polymorphisms are present in both the promoter regions and open reading frames.

Conclusions: Higher levels of expression of cdtA and its regulator in stationary phase compared to exponential phase is consistent with
the expression of tcdA and tcdB, suggesting common regulatory mechanisms. The wide range of expression levels between strains suggests a high level of heterogeneity even within C. difficile ribotypes. Heterogeneity is also observed in comparison of the CDT locus. The weak correlation between cdtA and its putative regulator cdtR suggest that while cdtR may have a regulatory role, other factors are probably also involved in cdtA expression. Further study of this, and the influence of exogenous factors such as antimicrobial exposure, is ongoing.

**P78** Preliminary molecular evaluation of the toxigenicity of *Clostridium difficile* strains isolated from dogs in the area of Parma (Italy)

M.C. Ossiprandi*, L. Zerbini (Parma, IT)

**Objectives:** *Clostridium difficile* has been associated with canine acute and chronic large and small bowel diarrhoea, as well as acute haemorrhagic diarrhoeal syndrome. Reports have documented a variable carriage rate of *C. difficile* ranging from 0–40% in diarrhoeic and non-diarrhoeic dogs. The purpose of this study was to evaluate the molecular characteristics of *C. difficile* strains isolated from diarrhoeic and non-diarrhoeic dogs by using PCR toxin gene profile.

**Methods:** Faecal samples were collected from 95 diarrhoeic and non-diarrhoeic dogs, tested for the presence of *C. difficile* toxins A/B with a commercially human EIA (Remel), and cultured onto pre-reduced selective medium before and/or after thermal shock. Preliminary identification of *C. difficile* was based on lack aerotolerance, colony appearance, odour, and cellular morphology following Gram staining. Species identities were confirmed through a rapid latex slide agglutination test (Oxoid) and Rapid ID32A (bioMérieux). All *C. difficile* isolates were PCR-screened for the presence of tcdA/tcdB and cdtA/cdtB genes, as previously described by Spigaglia and Mastrantonio (2002) and Stubbs (2000), respectively. Toxigenic strains were tested for in vitro toxin production by EIA.

**Results:** *C. difficile* strains were isolated from 10 of 95 canine faecal specimens (10.5%). Eight of the samples (80%) belonged to diarrhoeic dogs: 4 dogs were subjected to antibiotic treatment and the enteritis followed the therapy, 1 with megaoesophagus was treated for enteritis and *C. perfringens* was also isolated, 3 were not treated. The majority of *C. difficile* isolates (6/10, 60%) were toxigenic (tcdA+/tcdB+) and possessed ctdA and ctdB genes. All faecal samples tested by EIA were negative. On the contrary, all PCR-positive strains were positive for in vitro toxin production.

**Conclusion:** The results of this study suggest that commercially human EIA is inadequate for the diagnosis of canine *C. difficile*-associated diarrhoea when tested on faecal specimens, but it may be useful when used on toxigenic isolates. Moreover, based on our results, the isolation rates of *C. difficile* from diarrhoeic dogs (80.0%) and non-diarrhoeic dogs (20.0%) were statistically different. This is in disagreement with previous reports in which significant differences were not found in the isolation rates between the 2 groups. Probably, antibiotic administration caused the overgrowth of *C. difficile* in intestine of the dogs, predisposing the animals to enteritis.

**P78** Real-time PCR assays for the simultaneous detection of gyrA and gyrB mutations in *Clostridium difficile* isolates

P Spigaglia*, A. Carattoli, F. Barbanti, P. Mastrantonio (Rome, IT)

**Objectives:** Recent studies have demonstrated the involvement of fluoroquinolone (FQ) resistant *Clostridium difficile* strains in *C. difficile* infections (CDI). In particular, recent severe outbreaks were caused by the hypervirulent *C. difficile* PCR-ribotype 027/toxinotype III, a FQ resistant clone. In toxigenic strains, resistance to FQs is mainly associated with the amino acid change Thr to Ile in position 82 of GyrA and more rarely with the substitution Asp to Val in position 426 of GyrB. In this study, we developed two sets of primers and probes to be used in two single Real-Time PCR assays or in a multiplex assay for the detection of these substitutions.

**Methods:** Real-Time PCR assays were developed on the LightCycler Real-Time PCR platform (Roche Diagnostics). Single and multiplex assays were performed with the same reaction conditions. To distinguish between fluorescence emitted by each hybridisation probe set, two probes were labelled with a different fluorophore (LC-Red705 for gyrA and LC-Red640 for gyrB) and read in two different channels. The results were compared with MIC values obtained by the E-test and by sequencing the amplified genes.

**Results:** 17 toxigenic and 2 non toxigenic FQ resistant *C. difficile* strains isolated during the European prospective study performed in 2005 were used as representative of the different alleles of gyrA and gyrB currently known. Reference strain 630, susceptible to FQs, was used as control. Thirteen toxigenic strains showed the substitution in GyrA82 and 4 strains a substitution in GyrB426. The two non toxigenic isolates showed the substitution Arg to Lys in position 427 of GyrB (GyrB427). All strains with the substitution in GyrA82 showed a Tam of 52°C, compared to a Tam of 59°C of the wild type. Strains with the substitution Asp to Asn in GyrB426 showed a Tam of 53°C, strains with the substitution Asp to Val in GyrB426 a Tam of 54°C and the wild type and the strains with a change in GyrB427 a Tam of 59°C. A gyrB allele characterised by 3 silent mutations not involved in resistance showed a Tam of 50°C. The results were easy to interpret and always in agreement with those obtained by E-test and by sequencing.

**Conclusion:** These molecular assays for screening of gyrA and gyrB mutations are a reliable method for genetic detection of resistance to FQs in *C. difficile*, particularly in a setting where the use of these antibiotics may facilitate the dissemination of hypervirulent *C. difficile* strains.

**P78** Comparison of a multiplex real-time PCR and the cell cytotoxicity neutralisation assay for the diagnosis of *Clostridium difficile* infections

H. Huang, A. Weinstaab, H. Fang, C.E. Nord* (Stockholm, SE)

**Objectives:** *Clostridium difficile* infection (CDI) is the major cause of healthcare-associated diarrhoea. Recently, a new virulent *C. difficile* strain 027 causing outbreaks has emerged and was associated with increased morbidity and mortality. Therefore, rapid and accurate microbiological diagnosis is urgently needed. Toxigenic *C. difficile* detection by cell cytotoxicity neutralisation assay (CCNA) is considered to be the “gold standard”. However, this assay is time consuming, labour-intensive and requires facilities for cell culture. Toxin enzyme immunoassays are more rapid but are associated with widely varying sensitivity and specificity, making its reliability questionable for an accurate diagnosis of CDI. The purpose of this investigation was to evaluate the use of Xpert® C. difficile (Cepheid, Sunnyvale, CA) real-time multiplex polymerase chain reaction (PCR) assay as a diagnostic test for the detection of toxigenic *C. difficile* strains and presumptive ribotype 027.

**Methods:** A total of 125 unRepeated strains and 220 unformed fresh stools for *C. difficile* test were determined by CCNA. For the stool specimens, toxigenic cultures were performed additionally. All strains were typed by PCR-ribotyping. Concurrently, the Xpert® C. difficile Assay was also performed to identify toxin B (tdc B), binary toxin (tdc A/B), and the tdcC deletion at 117 (ribotype 027). The sensitivity and specificity of the Xpert® C. difficile Assay were determined related to CCNA and strain typing on the isolates.

**Results:** Of 125 strains, 17 (13.6%) were negative with both PCR and CCNA while 107 (85.6%) were positive with both assays yielding 100% sensitivity and 94.4% specificity. Of 220 stool specimens, 172 (78.2%) were negative with both PCR and CCNA, while 17 (7.7%) were positive with both assays yielding 97.1% sensitivity and 92.4% specificity. No ribotype 027 strain was found.

**Conclusions:** The Xpert® C. difficile Assay offers sensitivity and specificity that is comparable to the CCNA reference method. With the results available within one hour, it provides prompt and precise laboratory diagnosis and enables rapid and effective management of *Clostridium difficile* infections.
ST17 within CC17-E. faecium serves as substrate for sporadic acquisition of vancomycin resistance determinants despite dominance of ST18 among ampicillin resistant clones (Madrid, Spain)

P. Ruiz-Garbajosa*, G. Cárdenas, R. Cantón, F. Baquero, T.M. Coque (Madrid, ES, Quito, EC)

Objectives: Ampicillin-resistant E. faecium (AREfm) is associated with the expansion of a genetic lineage designated CC17. This CC grouped hospital-related isolates including most of the vancomycin resistant E. faecium (VREfm) causing hospital outbreaks. AREfm isolates have been suggested to serve as substrates for the emergence of VREfm. The aim of this study is to describe the population structure of VREfm isolates recovered in a setting with a low incidence of vancomycin resistance but a high incidence of ampicillin resistance and to analyze the corresponding glycopeptide resistance elements.

Methods: Eleven VREfm strains (8 VanB and 3 VanA) recovered at the Ramón y Cajal University Hospital (Madrid, Spain) (1996–2006) were studied. VREfm features were compared with a characterised collection of AREfm isolated from blood cultures (n=124) (1995–2008). VREfm strains were typed by MLST. The structural analysis of Tn1546 (VanA) and Tn1547(VanB) was assessed by a vanRSAYWHBX (11 kb) and vanRSYWHBX (6 kb) Long-PCR and PCR products were digested with Clal and BspHI/DraI respectively.

Results: Vancomycin resistance among E. faecium invasive and non-invasive isolates was 0.8% (15/1782) (1996–2006). In 11 years, VREfm were recovered from 15 patients (5 with VanA and 10 with VanB) admitted at surgery (40%) and medical (33%) wards and ICUs (27%). In 6/15 patients (40%) isolates were recovered from invasive samples (5 blood, 1 peritoneal fluid). All VREfm isolates were resistant to ampicillin and ciprofloxacin. By MLST, VREfm isolates were mainly grouped into ST17 (2 VanA and 5 VanB) and sporadically into ST16 (1 VanB), ST18 (2 VanB) and ST265 (1 VanA) all belonging to CC17. Among vancomycin susceptible-AREfm blood isolates, ST18 (n=62, 50%) was the most frequently found following by ST16 (n=17, 14%) and ST17 (n=17, 14%). Tn1546 and Tn1547 restriction profiles were identical in all VREfm strains and they corresponded with the complete backbone for these elements.

Conclusions: In our setting, VREfm strains were sporadically present. Vancomycin resistant determinants were infrequently acquired by endemic and persistent CC17-AREfm clones. Although endemicity of AREfm-ST18, vancomycin resistance appeared more frequently linked with ST17, suggesting differences in the ability for the acquisition in or the stability of these resistant determinants among endemic STs.

Vancomycin-resistant Enterococcus in Canada: results from the Canadian Nosocomial Infection Surveillance Program, 1999–2007


Objective: Surveillance is one component of a strategy to identify and limit the spread of Vancomycin Resistant Enterococci (VRE) in hospitals. The objective of our National surveillance system is to provide a Canadian benchmark rate for VRE.

Methods: The Canadian Nosocomial Infection Surveillance Program (CNISP) is comprised of 48 University-affiliated hospitals, including 8 paediatric stand-alone facilities in 9 Canadian provinces. Since 1999 surveillance for VRE has been ongoing. Cases of VRE are defined as inpatients from which Enterococcus faecium or Enterococcus faecalis having a minimum inhibitory concentration of vancomycin of >8 mg/mL is isolated from a clinical or screening specimen. Infection was defined as the presence of an illness that met standard infection surveillance definitions. Colonisation was defined as the presence of VRE in surgical wounds, urine, stool (rectum), or other body sites in an individual not manifesting clinical signs and/or symptoms. To be defined as healthcare-associated, there had to be no evidence that the organism was likely present at the time of admission.

Results: From 1999 to 2007, the rate of VRE increased from 0.37 to 2.48 cases per 1,000 admission s (do you mean, admissions). The increase in the rate was due primarily to an increase in VRE colonisation, from 0.34 to 2.74 cases per 1,000 admissions (p<0.0001). The rate of VRE infection increased from 0.02 to 0.08 cases per 1,000 admissions. The overall incidence of VRE increased from 1.20 per 1,000 admissions in 2006 to 2.48 per 1000 admissions in 2007 (p<0.0001), with increases seen in all regions of Canada. Most cases of VRE (82%) were health care-associated and were acquired in the reporting CNISP hospital. Overall, only 2% were community-acquired.

Conclusion: Although the incidence rate of VRE carriage in Canada is increasing, it remains low. There was a significant increase in the rate of VRE reported to CNISP in 2007 which more than doubled in a year. The number of cases of VRE acquired in the reporting CNISP hospitals increased in 2007 by 6%; whereas there was a decrease in the number of community-associated VRE of 6%.
**P793 Molecular epidemiology of Staphylococcus aureus among asymptomatic carriers from Saxony**

S. Monecke*, C. Luedicke, R. Ehrlich (Dresden, Jena, DE)

**Objectives:** The objective was to characterise the colonising *Staphylococcus aureus* population among asymptomatic carriers from Saxony to provide data for comparisons to isolates from defined clinical conditions.

**Methods:** Diagnostic microarrays were used in order to extensively characterise 155 *S. aureus* isolates obtained from asymptomatic carriers (admission screening of trauma and neurological patients, nasal swabs from junior medical students and from workers of a biomedical facility).

**Results:** Some superantigens proved to be very common. Toxic shock syndrome toxin (tst1) was detected in 14.8% of these 155 isolates. The enterotoxin cluster ege comprising of seg, sei, ser, see, and sec was very common (45.2%). Enterotoxin A (sea) was found in 17.4%. Enterotoxins D, J and R (sed, sej, ser) were always detected together in 15.5%. Enterotoxin genes C (sec) and L (sel) also occurred together in 12.3%. The genes encoding Panton-Valentine leukocidin ( lukS-PV and lukF-PV) were found only once, in a CC30 MSSA isolate. This virtual absence of PVL in asymptomatic carriers emphasizes its pathogenetic significance in patients with skin and soft tissue infections.

Three isolates (1.9%) were MRSA. Most isolates (71.6%) harboured the beta lactamase gene blaZ, while other resistance genes were found only sporadically. The 155 isolates typed in this study belonged to twenty different clonal complexes (CC). The most common CC was CC8 (18.7%). It was followed by CC15 (16.8%), CC30 (16.1%) and CC45 (9.9%).

**Discussion:** These data might provide an insight into pathogenesis, especially with regard to the different epidemiology of superantigens and PVL. In a previous study (Monecke & Ehrlich, 2007) PVL was found in 30% of abscess isolates, but it was present in only 0.6% of asymptomatic carriers. This emphasizes its pathogenetic significance in patients with skin and soft tissue infections. Contrastingly, there was virtually no difference between abscess and carrier isolates with regard to abundances of superantigens including tst1. Prevalence data on surface antigens, such as capsules, could be helpful for the design of a future vaccine.

**P794 Experience with polymerase chain reaction-based methicillin-resistant Staphylococcus aureus screening in paediatrics**

M. Patel*, J. Gray, H.L. Turner, J. Room, N.M. Dyer, S. Bullingham (Birmingham, UK)

**Objectives:** In England Department of Health Operational Guidance requires only that paediatric admissions in high-risk groups need to be screened. Our objective was to identify high-risk patients group based on previous 10-years experience of methicillin resistant *Staphylococcus aureus* (MRSA) in our hospital and investigate accuracy of polymerase chain reaction (PCR) based screening in high-risk patient group who might benefit from rapid availability of results.

**Methods:** In the 10-year period April 1998 to March 2007 MRSA was detected in 405 in-patients at Birmingham Children’s Hospital. A high proportion (20%) of the patients was from paediatric intensive care unit (PICU). PCR based screening was introduced for all admissions to PICU. A pair of nasal swabs was collected at the time of admission to the PICU. First swab was used for PCR using GeneXpert Dx system (Cepheid). The second swab was cultured on MRSA selective medium and then was placed in an MRSA enrichment broth and sub cultured onto MRSA selective medium after overnight incubation. The accuracy of PCR was determined by comparing PCR results with direct and enrichment culture of the nasal swab or any other clinical specimen growing MRSA at the same time.

**Results:** In two months period 203 specimens were processed from 180 patients. Thirteen nasal swabs were found to be positive by either PCR or culture. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of PCR when compared with culture results of screen specimens or clinical specimens was 88.9%, 97.9%, 66.7%, 99.5% and 97.5%, respectively (Table 1). We reviewed four PCR positive and culture negative cases. Two were direct hospital admissions from home with no known risk factors for MRSA and the other two were hospitalised and the positive result coincided with a culture confirmed case on the same unit.

**Results:** See Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Culture positive</th>
<th>Culture negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positive</td>
<td>8</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>PCR negative</td>
<td>1</td>
<td>190</td>
<td>191</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>194</td>
<td>203</td>
</tr>
</tbody>
</table>

**Conclusion:** In our study prevalence of culture confirmed MRSA in PICU patients was 5%. These data suggests that PCR may be more sensitive than direct culture. Significance of PCR positive and culture negative result is currently uncertain. Our preliminary data suggests that at least some of these results are true positives. We are now screening all PCR positive patients using swabs from multiple body sites to try to confirm patient’s MRSA status.

**P795 Development and implementation of a 4-plex real-time-PCR assay for screening and detection of methicillin-resistant and methicillin-sensitive Staphylococcus aureus**

S. Kolman, Y. Ben-Nissan, A. Kilman, C. Arieli, Y. Paitan* (Kfar Saba, IL)

**Objectives:** To develop a new real-time PCR (QR-PCR) assay for methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* (MRSA, MSSA) detection with high and clinically satisfactory diagnostic values (Sensitivity, Specificity, PPV, NPV) and to implement it in a cost-effective, logistically feasible protocol in our infection control program.

**Methods:** We developed a 4-plex QR-PCR assay enabling detection of MRSA and MSSA overcoming most problems found in previously published assays and in commercial MRSA kits. The assay simultaneously detects in one PCR tube, a PCR internal control (inhibition and reagents integrity), the mecA gene, a *S. aureus*-specific gene and SCCmec for the region types 1 to 5. The assay was validated using 150 known staphylococcal strains. Analytical specificity was analyzed using 83 different bacterial and fungal species. Analytical sensitivity, direct swab sensitivity and detection of MRSA and MSSA in mixed population were evaluated. All samples were analyzed for mixed population and by conventional identification methods. The assay was implemented in our infection control program in a cost-effective, logistically feasible protocol.

**Results and Diagnostic values for MRSA detection.**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (n=120)</td>
<td>95.4%</td>
<td>99.5%</td>
<td>99.3%</td>
<td>99.5%</td>
<td>99.5%</td>
</tr>
<tr>
<td>MRSA-negative (n=100)</td>
<td>95.4%</td>
<td>99.5%</td>
<td>99.3%</td>
<td>99.5%</td>
<td>99.5%</td>
</tr>
<tr>
<td>Calculated values</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PDIV</td>
<td>95.4%</td>
<td></td>
<td></td>
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<tr>
<td>NPVI</td>
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**Results and Diagnostic values for MSSA detection.**

<table>
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<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA (n=120)</td>
<td>99.5%</td>
<td>99.5%</td>
<td>99.5%</td>
<td>99.5%</td>
<td>99.5%</td>
</tr>
<tr>
<td>MSSA-negative (n=100)</td>
<td>99.5%</td>
<td>99.5%</td>
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<td>Calculated values</td>
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<td>PDIV</td>
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<td>NPVI</td>
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</table>

**Results:** Validation with 150 known staphylococcal strains revealed 100% concordance with microbiological analysis. No cross reaction
was observed to 83 different bacterial species (analytical specificity). The limit of detection was 20 CFU/PCR reaction for MRSA and 2 CFU/PCR for MSSA (analytical sensitivity). Direct swab sensitivity was 3000 CFU/ml for MRSA and 300 CFU/ml for MSSA corresponding to 150 CFU/swab or 20–25 CFU/PCR reaction for MRSA and to 15 CFU/swab or 2–4 CFU/PCR reaction for MSSA. No meaningful reduction was found in mixed population analysis. During 2 years, out of 39,120 samples, we applied the assay on 4,482 samples suspected to contain staphyloccoci or S. aureus in 504 runs (2500 samples after exclusion of patient’s duplicates). All calculated diagnostic values are ≈95–99% for MRSA detection and ≈91–99% for MSSA (Table 1). For logistical and economical reasons PCR is performed from colonies and results are available at the next day. Results are available in less then 3 hours if direct swab PCR is performed.

Conclusion: Our assay demonstrates very high diagnostics values, analytical sensitivity and specificity; suitable for direct swab analysis and can be incorporated into infection control programs in a cost-effective, logistically feasible protocol which will be presented.

**P796** Identification, cloning and characterisation of ScaD, a novel antigen from *Staphylococcus aureus*

M.R. Pournamdar*, S. Menbari, S. Foster (Tehran, IR; Sheffield, UK)

Objectives: *Staphylococcus aureus* and *Staphylococcus epidermidis* are major human pathogens of increasing importance due to the spread of antibiotic resistance. Novel potential targets for therapeutic antibodies are products of staphylococcal genes expressed during human infection. Sca gene family has been identified in both *S. aureus* and *S. epidermidis* with a highly conserved 110 amino acid C-terminal domain. ScaD is a novel protein demonstrating a strong identity with staphyloxanthin biosynthesis protein. In this study, purified recombinant protein was screened and analysed with patient sera.

Methods: ScaD has likely signal peptide, therefore for overexpression, the mature form of ScaD was produced. Restriction map of the scaD locus was created. It was found that Ndel and Xhol do not cut within the gene and so could be used for cloning. The pET21a(+) system was used for cloning and overexpression of the protein. The purified PCR fragment was digested with Ndel and Xhol, and ligated into pET21a(+) which had also been digested with the same enzymes. The recombinant plasmid was transformed into the expression host strain, *E. coli* BL21(DE3). The patient sera were collected form Tehran university of medical sciences hospitals.

Results: The mature *Staphylococcus aureus* ScaD was cloned and overexpressed successfully. Expression product of scaD exhibited a molecular weight by SDS-PAGE analysis comparable with a theoretical estimation. The soluble protein was tagged with 6xHIS allowing purification. SDS-PAGE and Western-blot analysis using patient sera demonstrated reactivity of the purified recombinant protein with patient sera.

Conclusion: ScaD belongs to the Sca protein family with a highly conserved C-terminal domain. Further studies to determine the role of the conserved ScaD domain in ligand binding and demonstration of conserved epitopes may establish the domain as a credible target for vaccination.

**P797** Evaluation of eSwab® for surveillance of MRSA by Xpert MRSA® and culture on pooled samples

K. Martens, H. De Beenhouwer, J. Frans*, A. Van den Abeele, R. Cartuyvels, G. Coppens (on behalf of the Biluta Study Group

Objectives: The Xpert MRSA® assay (Cepheid), which runs exclusively on the GeneXpert® system (Cepheid), is a FDA approved molecular test to screen for MRSA. It has previously been validated on nose, throat and perineum samples taken by a double Copan swab® (Copan). This study evaluates, in a multi-centre setting, the use of pooled eSwab® liquid transport medium from nose, throat and perineum (NTP) as input sample for the Xpert MRSA® assay in comparison to standard culture technique from the same medium.

Methods: High-risk patients (n = 159) were sampled from July until September 2008 in 5 Belgian hospitals. Separate nasal, throat and perineum swabs were collected using the eSwab®. Four hundred microl of each eSwab® liquid medium from a NTP set was pooled to a final volume of 1200 microl. For the molecular test using the Xpert MRSA® assay, 150 microl of pooled sample was added to the lysis buffer provided in the kit. Further testing was performed according to the manufacturer’s instructions. Another 500 microl of pooled sample was transferred to 4 mL of TSB and incubated for 18–24 h at 35°C. Ten microl of this enriched culture was transferred to a MRSA-ID® plate (bioMérieux) and screened for the presence of MRSA after 24 and 48 hours.

Results: Twenty nine (18.2%) samples were MRSA positive on culture. Of these 28 (96.6%) were Xpert MRSA® positive, while 1 (3.4%) tested negative. Of the 130 culture negative samples, Xpert MRSA® was negative in 125 (96.2%) but positive in 5 (3.8%) samples. Sensitivity and specificity of the Xpert MRSA® assay was 96.6% and 96.2%, respectively. The positive predictive value (PPV) was 84.8% and the negative predictive value (NPV) was 99.2%. No invalid results were observed for the Xpert MRSA® assay.

Conclusion: The results of Xpert MRSA® on eSwab® liquid transport medium pooled from NTP are comparable to the results previously obtained with the double Copan swab®, but fewer invalid results were obtained with eSwab®. The high NPV (99.2%) makes it suitable to rule out MRSA. However, due to the lower PPV (84.8%), positive Xpert MRSA® results need confirmation by culture. For culture, the same eSwab fluid can be used, reducing the risk of sampling bias. Pooled eSwab® liquid medium from NTP is an adequate matrix for rapid MRSA screening by the Xpert MRSA® assay with culture confirmation possibility without extra sampling.

Table 1: Overview of the results performed on the GeneXpert® system and eSwab® culture method

<table>
<thead>
<tr>
<th>eSwab® culture +</th>
<th>eSwab® culture –</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneXpert® +</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>GeneXpert® –</td>
<td>1</td>
<td>125</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>130</td>
</tr>
</tbody>
</table>

**P798** Sequence-based characterisation of the agrD gene and the hld gene in *Staphylococcus epidermidis* isolated from prosthetic joint infections

B. Hellmark*, B. Söderquist, M. Unemo, Å. Nilsdotter-Augustinsson (Orebro, Linkoping, SE)

Objectives: *Staphylococcus epidermidis* is a commensal that comprises a substantial part of the normal human skin flora. Nevertheless, this bacterium has emerged as the most important pathogen in infections related to implanted foreign body materials, especially prosthetic joint infections (PJJIs). A regulatory gene-complex, called accessory gene regulator (agr), has a major role in the regulation of different virulence factors and it has been found to be polymorphic. It consists of four genes; agrA, agrB, agrC, and agrD. By sequencing of the agrD gene, three different agr types have been described and there seems to be a correlation between agr type and the virulence of the bacteria. One of the regulated virulence factors is the delta toxin, which is encoded by the hld gene. The hld gene is located in close proximity of the agr complex. The aim of this study was to sequence the agrD gene and the hld gene in *S. epidermidis* isolated from PJJIs, and compare the sequences with the agrD gene and the hld gene in commensal *S. epidermidis*.

Methods: Thirty-three *S. epidermidis* isolates obtained during revision surgery due to PJJIs were analyzed. Twenty-four commensal *S. epidermidis* isolates from 24 healthy individuals were used as controls, 12 from skin and 12 from nares. Sequencing of the agr complex and the hld gene was used.
Results: Among the 33 isolates from PJs all three agr types were identified; 18 isolates displayed agr type 1, two type 2, and 13 type 3. Among the 24 commensal isolates 10 isolates comprised agr type 1, three type 2, and seven type 3. Furthermore, two isolates displayed an AgrD amino acid sequence that was similar to agr type 2 but had one and two amino acid alterations, respectively. Two isolates were not possible to type due to lack of PCR amplicons. In nearly all evaluated PJ (33/33; 100%) and commensal (21/22; 96%) isolates, the hld gene was present and encoded identical amino acid sequence. Accordingly, one commensal isolate displayed a one amino acid alteration and in two commensals the presence of the hld gene could not be evaluated due to lack of PCR amplicons.

Conclusions: Regarding the correlation between agr type and origin of isolates there was no statistically significant difference; type 1 was the most common type in isolates from both PJs and the commensals. In addition, no difference in prevalence of the hld gene between the two groups of isolates was found.

P799 Genotypic characterisation of Helicobacter pylori isolates from southern Poland
E. Karczewska, I. Wojtas, A. Bulak*, E. Sito, M. Zwołinska-Wcisło (Cracow, PL)

Objectives: The high level of Helicobacter pylori infection in Poland (about 60%) may constitute a risk factor of still high morbidity rate on gastrointestinal diseases in this country. H. pylori strains that possess the vacA s1/m1 (or s1/m2) cagA-positive genotypes have been associated with more severe clinical outcomes. The aim of the present study was to determine the prevalence of the cagA gene and the vacA alleles in H. pylori strains isolated from dyspeptic patients and to investigate the correlation of the vacA genotypes with cagA status as well as the relation between the cagA and vacA genotypes and different clinical outcomes.

Methods: The vacA (s1, s2, m1, m2) and cagA genotypes of 46 H. pylori isolates from patients in the southern part of Poland were characterised by PCR. The prevalence of the different cagA and vacA genotypes in two clinical groups, peptic ulcer disease (PUD) and non ulcer dyspepsia (NUD) was compared and the correlation between the distinct genotypes and clinical outcomes was statistically examined.

Results: Thirty (65%) H. pylori strains were identified as cagA-positive. The three of four possible combinations of the vacA signal (s) and middle (m) regions were identified in this population and the most frequent genotypes were s1/m1 and s1/m2 (both 37%). The s2/m2 genotype was strongly associated with the cagA-negative strains (p <0.001). The presence of the vacA s1 genotype correlated with the cagA-positive isolates (p<0.001) and the m2 isolates were associated with the cagA-negative strains (p=0.012). There were no statistically significant differences in the prevalence of the cagA and vacA genotypes in the groups of patients with PUD and NUD, but considering these groups separately the presence of the s1 allele was significantly associated with PUD as well as with NUD (p <0.001, p =0.007, respectively), the m2 allele – with NUD (p=0.027) but not with PUD (p=0.064). The cagA gene displayed an association with PUD (p<0.001) in contrast to NUD (p=0.2).

Conclusion: Our study demonstrates that individuals infected with H. pylori strains that carry the vacA s1 cagA-positive genotypes are associated with increased risk of PUD, compared to those infected with the vacA s2 cagA-negative strains. It can mean that some non ulcer dyspepsia patients with the vacA s1 cagA-positive strains will be at risk of developing PUD in the future.

P800 Identification of sialic acid biosynthesis and transferase locus in human and canine Helicobacter bizzozeronii strains
M. Rossi*, T. Raatila, M. Hänninen (Helsinki, FI)

Helicobacter bizzozeronii is a canine gastric species belonging to the “Helicobacter heilmannii” Type 2. “H. heilmannii” comprises at least five different Helicobacter species observed occasionally (0.17–2.3%) in gastric biopsies of human patients with upper gastrointestinal symptoms. These organisms are difficult to cultivate in vitro and H. bizzozeronii is the only “H. heilmannii” species isolated from human. Up to now, only two human H. bizzozeronii strains are available, one isolated from a Danish patient and the other from a Finnish one. The genome of H. bizzozeronii CCUG 35545T was sequenced using the 454-pyrosequencing technology. Preliminary gene prediction and homology searches for each contig larger than 1000 kb were carried out. A cluster of three predicted genes, 4899 bp in total length, presenting high homology to genes involved in the synthesis of sialylated lipopolysaccharide outer core in Campylobacter jejuni (neuB, neuC, neuA and two copies of csII) were identified. The G+C% of this island was equal to the genome average value of 46% and showed identical synteny and high levels of homology to a cluster of genes located downstream of the fragmented vacA locus of Helicobacter acinonychis strain Sheeba. Homopolymeric runs of 15 residues of cysteine and 15 of guanine were detected upstream and downstream of the first csII homologue suggesting a potential site of phase-variation in H. bizzozeronii. A PCR primer set was designed in order to amplify the spanning region between neuA and the first csII homologues of four canine and two human strains. No fragments were amplified from the Finnish human strain and from one canine isolate, indicating either the absence of the island or a change in the synteny. The 354 bp fragments of the Danish human strain and of one canine strain showed the expected repeat of cytosine and 96.5% of identity with the sequence of H. bizzozeronii CCUG 35545T, whereas two canine strains lacked in the poly-C stretches and showed less identity with the type strain (92.4% and 94.6%). Further characterisation of the entire fragment is still in process. The role of this island in H. bizzozeronii lipopolysaccharide biosynthesis or protein glycosylation as well as the function of the high plasticity observed among human and dog strains in the host adaptation are the aims of our further studies.

P801 Helicobacter pylori and Candida albicans: interrelation and genetic features of micro-organisms in patients with duodenal ulcer
N. Baryshnikova, E. Tkachenko, Y. Uspenskiy, A. Saurov* (St. Petersburg, RU)

Objective: There are a lot of the evidences supporting the direct correlation between the presence of Helicobacter pylori (HP) in a stomach and Candida albicans (CA) in colon (Zaharchenko M.M., 2003, Baryshnikova N.V., 2006). There were researches in which yeast (fungi) are considered as HP carriers (Siavoshi F et al., 2005). The aims of research are revealing interrelation between the presence of HP and CA in stomach mucosa and investigating the genetic features of these microorganisms in patients with duodenal ulcer (DU).

Materials and Methods: 27 DU patients were included in this research. All patients were treated with standard eradication therapy (omeprazole, amoxicillin, clarithromycin) during 10 days. An antifungal treatment was not appointed. Before and after therapy polymerase chain reaction (PCR) in biopsy material of stomach mucosa was performed for all patients. Several genes of pathogenicity island (PAI) of HP (ureC, cagA, cagC, cagA, cagH) and genes of CA (sap2 – secreted aspartic proteases, hwp1 – hyphal wall protein 1, alp7 – agglutinin like protein 7) were studied.

Results: It was determined, that at all DU patients carried both CA and HP before treatment. The HP genes encoding for PAI were determined quite often: cagA – at 88.9%, cagC – at 77.8%, cagE – at 77.8%, cagH – at 77.8% of patients. The combination of all four above-named genes was found in 92.6% of cases. CA genes sap2 and alp7 were determined in 100% of patients, gene hwp1 – in 51.9%. After the anti-HP-therapy the percentage of determining of hwp1 gene decreased by 25%. To other CA genes frequency didn’t change. The proportion of successful HP eradication was 66.7%, and in this group of patients the gene hwp1 of CA was identified by PCR-method in 44.4% of cases before treatment and only in 22.2% – after therapy (p<0.05).

Conclusions: Highly virulent strains of Helicobacter pylori can be often associated with carriage of Candida albicans. Anti-HP-therapy can significantly decrease the carriage of CA in stomach mucosa. Possibility mechanisms of this phenomenon in discussed.
**P802** Comparison of Genotype® HelicoDR with real-time PCR to identify clarithromycin resistance in *Helicobacter pylori*

S. Agudo*, T. Alarcón, P. Urruzuno, A. Somodevilla, M. López-Brea (Madrid, ES)

**Objective:** Clarithromycin is one of the most important antibiotics for *Helicobacter pylori* treatment and it is an important factor of eradication failure. The early detection of resistance is important in the treatment of *H. pylori*. The aim of this study was to evaluate two commercially available kits: the MutaREAL® *H. pylori* Real Time PCR Kit (MutaREAL Immundiagnostik), and a assay based on DNA hybridisation technology on nitrocellulose strips, Genotype® HelicoDR (Hain, Diagnostika, Nehren, Germany) for detection of point mutations in the 23S rRNA genes responsible for *H. pylori* clarithromycin resistance in gastric biopsies.

**Methods:** A panel of 126 *H. pylori* strains was studied for phenotypic (MIC) and genotypic resistance to clarithromycin (rrl mutation) and levofloxacin (gyrA mutation). Genotype correlated to phenotype at the rate of 97% for clarithromycin and 100% for levofloxacin. On the basis of the panel test sequencing results and literature data, we developed a genotyping test using the DNA Strip technology. Oligonucleotide probes were wild-type sequences or sequences of the most prevalent mutations. Twenty *Helicobacter* species other than *H. pylori* were tested to assess analytical specificity. Clinical strains (n=64) and *H. pylori* positive gastric biopsies (n=109) were tested blindly with the new molecular test GenoType® HelicoDR.

**Results:** The presence of mutations or the absence of hybridisation with wild-type sequences was predictive in rrl, for clarithromycin resistance in 85 cases (mostly the A2147G mutation), and in gyrA for levofloxacin resistance in 53 cases (mutations at codons 87 or 91). Sensitivity, specificity, and positive and negative predictive values for detecting resistance were 95.5%, 96.5%, 98%, and 96.5% for clarithromycin, respectively, and 87%, 99%, 98% and 93% for levofloxacin, respectively. Concordance score was 0.97 for clarithromycin and 0.95 for levofloxacin. Genotyping showed a mix of genotypes reflecting a co-infection in 31% of strains and 35% of biopsies.

**Conclusion:** GenoType® HelicoDR is an efficient method to detect mutations predictive of antibiotic resistance in *H. pylori* when applied to strains or directly to gastric biopsies.

**P804** Analysis of 3’ end variable region of cagA in *Helicobacter pylori* isolated from Iranian dyspeptic patients


**Objective:** CagA protein, encoded by the cagA, is the best studied virulence factors of *H. pylori*. CagA is mainly tyrosine-phosphorylated at Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs. The aim of the present study was to investigate the structures of the 3’ region of the cagA gene, number and type of EPIYA motifs in Iranian *H. pylori* isolates.

**Methods:** A total of 190 gastric biopsies from patients with dyspeptic symptoms who were qualified for endoscopies of upper gastrointestinal tract from February 2007 to February 2008 were included in this study. Samples were cultured by standard methods and genomic DNA was extracted by using the QIAamp tissue DNA extraction kit. After confirming isolates with glmM followed by amplifying cagA gene, we examined 92 cagA gene-positive *H. pylori* isolates. The entire 3’ end variable region of the cagA gene was amplified by PCR followed by sequencing.

**Results:** Out of 92 *H. pylori* cagA-positive isolates, the EPIYA motif was present in 86 strains with three copies, 3 strains with four copies, and 3 strains with two copies. Sequence analysis of the entire 3’ end PCR products showed three types of primary gene structure depending on the type and number of repeats: EPIYA-ABC, EPIYA-ABCC and EPIYA-AB. We found no strains within our population harbouring the Eastern type of EPIYA-D.

**Conclusion:** Studies of the structure of the 3’ region of the cagA gene of *H. pylori* isolates in Iran as an East country showed that this region of cagA differs markedly from Eastern isolates. On the other hand, alignments of deduced amino acid showed that the type of Iranian isolates can be related to Western types rather than Eastern types. Differences in cagA 3’ region can be useful for molecular epidemiological studies and it may provide a marker for differences in virulence among cagA-positive *H. pylori* strains.

**P803** Development of a new test, GenoType® HelicoDR, for molecular detection of antibiotic resistance in *Helicobacter pylori*

E. Cambau*, V. Allerheiligen, C. Corbel, L. Deforges, F. Megraud (Paris, Bordeaux, FR, Nehren, DE)

**Objectives:** Eradication rate of *Helicobacter pylori* by standard therapy is decreasing due to an increase in antibiotic resistance. Our aim was to provide a new molecular test to facilitate detection of this resistance.

**Methods:** A panel of 126 *H. pylori* strains was studied for phenotypic (MIC) and genotypic resistance to clarithromycin (rrl mutation) and levofloxacin (gyrA mutation). Genotype correlated to phenotype at the rate of 97% for clarithromycin and 100% for levofloxacin. On the basis of the panel test sequencing results and literature data, we developed a genotyping test using the DNA Strip technology. Oligonucleotide probes were wild-type sequences or sequences of the most prevalent mutations. Twenty *Helicobacter* species other than *H. pylori* were tested to assess analytical specificity. Clinical strains (n=64) and *H. pylori* positive gastric biopsies (n=109) were tested blindly with the new molecular test GenoType® HelicoDR.

**Results:** The presence of mutations or the absence of hybridisation with wild-type sequences was predictive in rrl, for clarithromycin resistance in 85 cases (mostly the A2147G mutation), and in gyrA for levofloxacin resistance in 53 cases (mutations at codons 87 or 91). Sensitivity, specificity, and positive and negative predictive values for detecting resistance were 95.5%, 96.5%, 98%, and 96.5% for clarithromycin, respectively, and 87%, 99%, 98% and 93% for levofloxacin, respectively. Concordance score was 0.97 for clarithromycin and 0.95 for levofloxacin. Genotyping showed a mix of genotypes reflecting a co-infection in 31% of strains and 35% of biopsies.

**Conclusion:** GenoType® HelicoDR is an efficient method to detect mutations predictive of antibiotic resistance in *H. pylori* when applied to strains or directly to gastric biopsies.

**P805** Frequency of vacA, cagA and babA2 virulence markers in *Helicobacter pylori* strains isolated from Mexican patients with chronic gastritis


**Objective:** Helicobacter pylori has been strongly associated with chronic gastritis, peptic and duodenal ulcers, and is a risk factor for gastric cancer. Three major virulence factors of *H. pylori* have been described: the vacuolating toxin (VacA), the cytotoxin-associated gene product (CagA) and the adhesion protein BabA2. Since considerable geographic diversity in the prevalence of *H. pylori* virulence factors has been reported, the aim of this work was to establish the *H. pylori* and vacA, cagA and babA2 gene status in one hundred adult patients, from a marginal urban area of Mexico, with chronic gastritis.

**Methods:** *H. pylori* was identified in cultures of gastric biopsies by nested PCR, vacA and cagA genes were detected by multiplex PCR, whereas babA2 gene was identified by conventional PCR.

**Results:** *H. pylori*-positive biopsies were 81%. All *H. pylori* strains were vacA-: 38.3% were cagA+; 21% were cagA- babA2+ and 6.2% were babA2-. Mexican strain examined possessed the vacA s1, m1 (40.7%), s2, m1 (28.4%), s2, m1 (16%) and s2, m2 (14.8%) genotypes.
Conclusion: These results show that the Mexican patients suffering chronic gastritis we have studied had a high incidence of infection by *H. pylori*. Most *H. pylori* strains examined may be considered as highly virulent since sixty six percent of them possessed two or three of the virulence markers analyzed, with s1, m1 as the most frequent alleles of vacA.

Acknowledgements: Project supported by PAPIIT IN216508, DGAPA, UNAM.

Antimicrobial susceptibility testing and resistance detection

**[P806] EUCAST breakpoints in automated susceptibility testing of Gram-negative bacteria – BD Phoenix validated**

R. Smyth*, S. Bengtsson, G. Kahlmeter, G. Babini, E. Montrucchio (Växjö, SE; Buccinasco, IT)

Objectives: To evaluate the performance of the BD Phoenix AST System for susceptibility testing of Gram negative clinical isolates and their SIR-category interpretation using breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Methods: 242 Gram negatives, both stored strains and fresh isolates, were tested in parallel using BD Phoenix (PHX) and the Swedish Reference Group for Antibiotics (SRGA) disc diffusion method. The PHX panels, specifically manufactured for this study, were NMIC/ID-73, containing amikacin (AN), amoxicillin/clavulanate (AXC), aztreonam (ATM), cepheime (FEP), cefotaxime (CTX), cefazidime (CAZ), cefuroxime (CMX), ciprofloxacin (CIP), colistin (CL), gentamicin (GM), imipenem (IPM), meropenem (MEM), moxifloxacin (MXF), nalidixic acid (NA), piperacillin (PIP), piperacillin/tazobactam (TZP), tobramycin (NN), trimethoprim (TMP) and trimethoprim/sulphamethoxazole (SXT), each in an appropriate range of doubling dilutions. Category (SIR) interpretations of MIC results from PHX and inhibition zone results from disc diffusion were in line with EUCAST breakpoints. Discrepancies were resolved by retesting and then if necessary by MIC determination using E-test. Reference strains *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603 were included for QC on each day of testing.

Results: The results are summarised in the table. Overall category agreement was 98.8% for 3444 tests. A variety of ESBL producers, 30 strains in all, were tested. PHX detected all but one, a CTX-M 14 conferring low-level resistance (CTX MIC 2 mg/L). Among the 36 strains of *P. aeruginosa*, 7 major errors (ME) were seen for FEP, one ME for IPM and two minor errors (mE) for CIP and MEM. For *E. coli*, one ME for AXC and SXT, one mE for each of CTX, TZP, CAZ, GM and NN, one very major error (VME) for NA.

Conclusion: This first evaluation of EUCAST breakpoints in an automated system for Gram negative bacteria indicates PHX to be a reliable tool.

<table>
<thead>
<tr>
<th>Organism group</th>
<th>CA first test</th>
<th>CA final test</th>
<th>Total tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>305</td>
<td>94.1</td>
<td>314</td>
</tr>
<tr>
<td>ESBL</td>
<td>443</td>
<td>92.3</td>
<td>463</td>
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<tr>
<td><em>Esch. coli</em></td>
<td>937</td>
<td>97.6</td>
<td>952</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>652</td>
<td>98.8</td>
<td>660</td>
</tr>
<tr>
<td><em>K. oxytoca</em></td>
<td>176</td>
<td>97.8</td>
<td>180</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>321</td>
<td>97.3</td>
<td>328</td>
</tr>
<tr>
<td><em>M. morganii</em></td>
<td>176</td>
<td>97.8</td>
<td>179</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>172</td>
<td>95.6</td>
<td>177</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>147</td>
<td>98.0</td>
<td>149</td>
</tr>
<tr>
<td>Total</td>
<td>3329</td>
<td>96.7</td>
<td>3402</td>
</tr>
</tbody>
</table>

**[P807] EUCAST breakpoints in automated susceptibility testing of Gram-positive bacteria – BD Phoenix validated**

R. Smyth*, S. Bengtsson, G. Kahlmeter, G. Babini, E. Montrucchio (Växjö, SE; Buccinasco, IT)

Objectives: To evaluate the performance of BD Phoenix AST System for susceptibility testing of Gram positive clinical isolates and their SIR-category interpretation using breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Methods: 280 clinical isolates of known identity comprising 114 staphylococci, 25 enterococci and 141 streptococci, both stored strains and fresh isolates, were tested in parallel using BD Phoenix (PHX) and the Swedish Reference Group for Antibiotics (SRGA) disc diffusion method.

The PHX panels, specifically manufactured for this study were PMIC/ID-66, containing ampicillin, cefoxitin, ciprofloxacin, clindamycin, daptomycin, erythromycin, fusidic acid, gentamicin, imipenem, linezolid, moxifloxacin, nitrofurantoin, oxacillin, penicillin, quinupristin-dalfopristin, rifampicin, teicoplanin, tetracycline and vancomycin, each in an appropriate range of doubling dilutions. Category (SIR) interpretations of MIC results from PHX and inhibition zone results from disc diffusion were in line with EUCAST breakpoints. Discrepancies were resolved by retesting and then if necessary by MIC determination using E-test. Reference strains *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619 and *S. aureus* ATCC 29213 were included for QC on each day of testing.

Conclusion: This is the first evaluation of EUCAST breakpoints for Gram positive organisms in an automated system and the results indicate PHX to be a reliable tool.

<table>
<thead>
<tr>
<th>Organism group</th>
<th>CA first test</th>
<th>CA final test</th>
<th>Total tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
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<tr>
<td>MRSA</td>
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<tr>
<td>MSSA</td>
<td>888</td>
<td>99.1</td>
<td>893</td>
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<tr>
<td>CoNS</td>
<td>353</td>
<td>98.1</td>
<td>353</td>
</tr>
<tr>
<td><em>S. lugdunensis</em></td>
<td>108</td>
<td>100.0</td>
<td>108</td>
</tr>
<tr>
<td><em>Str. pneumoniae</em></td>
<td>256</td>
<td>99.2</td>
<td>258</td>
</tr>
<tr>
<td><em>Str. pyogenes</em></td>
<td>244</td>
<td>99.6</td>
<td>244</td>
</tr>
<tr>
<td><em>Str. agalactiae</em></td>
<td>244</td>
<td>99.5</td>
<td>244</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>141</td>
<td>100.0</td>
<td>141</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>109</td>
<td>97.3</td>
<td>109</td>
</tr>
<tr>
<td>Total</td>
<td>2513</td>
<td>98.9</td>
<td>2524</td>
</tr>
</tbody>
</table>


E. Matuschek*, R. Smyth, G. Kahlmeter (Växjö, SE)

Objective: There are several methods available for antimicrobial susceptibility testing in Europe, but no European standard disc diffusion method. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) was recently tasked with developing a European disc diffusion method calibrated against the recently harmonised European MIC breakpoints. This should preferably be achieved in 2009. This poster
describes the basic details of the proposed new European method and presents tables of target zone diameters and ranges for quality control strains. Where relevant, the EUCAST and Clinical and Laboratory Standards Institute (CLSI) methods are compared.

Methods: The method is based on two media, Mueller-Hinton agar (MHA) without supplements (medium A) for non-fastidious organisms and MHA with 5% horse blood and 20 mg L−NAD/L (medium B) for streptococci, Haemophilus and other fastidious organisms. Medium A was incubated in air 18±2 h and medium B in 5% CO2 for 18±2 h. The inoculum suspension was 1:105 CFU/mL, corresponding to McFarland 0.5 for all organisms except for S. pneumoniae (McF 1.0). For non-fastidious organisms the EUCAST and CLSI methods are in almost all respects identical. The EUCAST method does, however, suggest a common medium for fastidious organisms, while the CLSI method requires two different media for these organisms. In the present study, inhibition zone diameters were obtained with the EUCAST disc diffusion test for a large number of agents, for several quality control strains and on five batches of MHA from three producers.

Results: All microorganisms tested showed satisfactory growth after 16–20 hours on the five tested batches of medium. Disc diffusion testing for a battery of antimicrobial agents resulted in reproducible inhibition zone sizes (mean ± SD mm) of 12 ± 1 mm. For most organisms, there were differences between the CLSI and EUCAST results.

Conclusion: A new European standard method for antimicrobial susceptibility testing is being developed by EUCAST. Preliminary results show that non-supplemented MHA and MHA with 5% horse blood and 20 mg L−NAD/L can be used for non-fastidious and fastidious organisms, respectively.

Comparison of results from testing sensitivity to amoxicillin/clavulanate with CLSI versus EUCAST interpretation

M. Hoeck*, B. Wiedemann (Berlin, Schaalby, DE)

Objectives: Methods and interpretation criteria for susceptibility testing with amoxicillin/clavulanate differ markedly for CLSI and EUCAST method. By using both methods in parallel we wanted to analyse the difference in the results.

Methods: In one laboratory of the EPICENTER network both CLSI and EUCAST combination types of amoxicillin/clavulanate were used in parallel to determine the MICs for E. coli and Klebsiella pneumoniae with the BD PHOENIX system. The CLSI combination of amoxicillin/clavulanate was tested at the concentrations 1/2, 2/2, 4/2, and 16/8. The concentration of EUCAST combination were 1/2, 2, 2/2, and 8/2. The criteria for interpretation were for CLSI: S ≤ 8, R ≥ 32, for EUCAST: S ≤ 8, R ≥ 16. In addition we used the DIN breakpoints S ≤ 2, R ≥ 16, because EUCAST does not recommend a fixed “S” breakpoint because of the different possibilities for dosing and application.

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>Klebsiella pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLSI</td>
<td>EUCAST</td>
</tr>
<tr>
<td>% Resistant</td>
<td>6.7</td>
<td>39.8</td>
</tr>
<tr>
<td>% Sensitive</td>
<td>77.2</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Results: 16963 E. coli – and 5037 K. pneumoniae – isolates were tested between 2003 and 2008 and included in the analysis. The MIC distributions with both methods have different shapes. While the EUCAST method gives a bimodal distribution with the resistant strains ≥ 16 mg/L, the CLSI method gives more or less a unimodal distribution for E. coli and Klebsiella. The relative interpretive results are given in the table. Most striking is the difference in the number of resistant strains for E. coli: 6.7 for the CLSI method versus nearly 40% with the EUCAST method. In addition, for scientific interest, we interpreted the results of the CLSI combination with EUCAST criteria and vice versa what is obsolete in the daily practice but unfortunately in few laboratories is still common practice. The differences were less evident.

Conclusion: Microbiologists and clinicians should be aware of the differences in the methods and interpretation of test results the comb of the combination of amoxicillin with clavulanate. We believe the EUCAST method to be more on the safe side.

Comparison of the M.I.C.Evaluator™ (Oxoid M.I.C.E) and ETest® (AB Biodisk – BioMérieux) for antimicrobial susceptibility testing of anaerobic bacterial species

L.A. Turnbull*, C. Brunoni, R. Rennie (Edmonton, CA)

Objectives: Historically only one gradient end point product has been available for the routine antimicrobial susceptibility testing of common aerobic and anaerobic bacteria. This study was developed to compare a new agar gradient end point method (M.I.C.ETM-Oxoid; Thermo Fisher Scientific). For routine testing, such devices are important for anaerobes that cannot be tested on automated systems.

Methods: A total of 102 recently isolated strains comprising of 28 species of anaerobes were tested. Four agents were tested: amoxicillin-clavulanate (AMC), imipenem (IPM), metronidazole (MTZ) and penicillin (P) (low and high concentration strips). Agar dilution tests were also performed on brucella agar supplemented with 5% laked sheep blood, haematin, and vitamin K according to CLSI guidelines. ETest was also tested. Strips for each agent were added to the plates according to manufacturers’ instructions and the plates were incubated for 24–72 h at 350C in an anaerobic atmosphere. Inhibition for each strip was read at the point where the elliptical zone intersected with the strip. Quality control strains for these tests were Bacteroides fragilis ATCC® 25285TM, and Bacteroides thetaiotaomicron ATCC® 29741TM. Performance was evaluated using FDA criteria.

Results: All quality control results for all tests were within CLSI published ranges. For clinical strains, agreement between M.I.C.E and ETest was excellent. Essential and categorical agreement (EA & CA) between the two devices was 98% and 100% respectively for P, MTZ and IPM. For AMC, EA was lower (90%) but CA was 95%. Agar dilution results for clinical isolates did not correlate well with either M.I.C.E or ETest. Essential agreements were less than 90% for all four agents. Using agar dilution as a standard, there were 14% very major errors for MTZ, and 9% for P. There were less than 1% major or very major errors for AMC and IPM when M.I.C.E and ETest were compared to agar dilution.

Conclusions: M.I.C.E and ETest results for 102 clinical strains of anaerobes were in essential and categorical agreement. Quality control results with the strip tests and for agar dilution were in range, but there were differences between clinical isolates when the strips were compared to agar dilution. The consistency between M.I.C.E and ETest results for these clinical strains suggests that this methodology provides an easy, rapid and reproducible means of determining antimicrobial susceptibility for most anaerobe species.

Comparative evaluation of four commercial methods for antimicrobial susceptibility testing of a collection of 96 Aeromonas clinical isolates


Commercial systems offer the possibility to evaluate susceptibility of Aeromonas strains, although this has never been extensively evaluated.

Objectives: To evaluate accuracies of 4 commercial methods for antimicrobial susceptibility testing for 96 clinical Aeromonas strains.

Methods: Antimicrobial susceptibility testing with API ATB G-strips (A) (API, bioMérieux, France), AST NO52 card (B) (VITEK2,
Appropriate Muller-Hinton agar is crucial for the performance of metallo-β-lactamase E-test

N. Al Naimi*, Y.J. Dehets-Ossenkopp, P.S. Lee, P. Saevkoul, C. Vandenbroucke-Grauls (Amsterdam, NL)

Objectives: Metallo-β-lactamases (MBL)-producing bacteria form a real challenge to routine microbiology laboratories, as there are no standardised methods for detection of these multiresistant isolates. During an MBL prevalence study performed at our hospital, we noticed that 6 of 9 positive MBL E-test results were false positive. We investigated whether this high percentage of false positive results was due to the use of inappropriate Muller-Hinton (MH) agar.

Methods: The presence of carbapenemases was assessed by PCR with primers for blaIMP, blaVIM, blaGIM, and blaSPM, and by cloning and sequencing. The performance of MBL E-test was evaluated with different MH agars with A. baumannii harbouring armA and its transconjugant.

Results: Of the 9 phenotypically MBL-positive strains (E-test with Difico MH agar), three isolates were confirmed MBL-positive by PCR and sequencing. The three MBL-producing isolates were detected correctly with E-test performed on all MH agars except on bioMérieux MH agar, which showed false resistance. While the 6 MBL-negative isolates were detected correctly with bioMérieux MH agar, the number of false positive E-tests on other MH agars was the following: Becton Dickinson MH: 1; Oxoid: 2; and Difico: 0.

Conclusions: These data showed that appropriate MH agar is crucial for the performance of MBL E-test and emphasized the necessity for standard guidelines for detection of these multiresistant bacterial strains.

P813 False susceptibility to amikacin by VITEK 2 in Acinetobacter baumannii harbouring armA


Objectives: Amikacin (AN) is the most active aminoglycoside for treatment of infections caused by Acinetobacter baumannii. The VITEK 2 (bioMérieux Inc., Hazelwood, MO) automated system recommends an alternative susceptibility testing prior to reporting of AN susceptibility result for A. baumannii. We accidently found an A. baumannii isolate which was susceptible to AN by VITEK 2, but showed resistance by disk diffusion test and agar dilution MIC test.

Methods: On June 2008, we performed a disk diffusion test for an A. baumannii isolate which was susceptible to AN by VITEK 2. AN MIC was determined by agar dilution method according to the CLSI guideline and presence of 16S rRNA methylase genes were investigated by PCR. Also, we tested the AN susceptibility of Serratia marcescens isolate harbouring armA and its transconjugant (E. coli 153 harbouring armA) by VITEK 2, disk diffusion test and agar dilution method. To check the purity, we picked a single colony from the A. baumannii and E. coli transconjugant into MH broth, and repeated the disk diffusion test. In addition, to investigate whether this phenomenon is associated with induction, we assayed the resistant subpopulations directly for AN susceptibility.

Results: A. baumannii showed susceptibility to AN by VITEK 2 but revealed resistance (double zone of inhibition) by disk diffusion test. AN MIC was >512 µg/ml and it was harbouring armA. In cases of S. marcescens harbouring armA and its transconjugant (E. coli 153 harbouring armA), S. marcescens showed resistance to AN but its transconjugant showed susceptibility to AN by VITEK 2. By disk diffusion test, the S. marcescens and its transconjugant revealed complete resistance and resistance with double zone of inhibition, respectively. Both A. baumannii and E. coli showed same phenomenon with a single colony. However, the AN MICs of the two isolates were both very high (>512 µg/ml). The direct susceptibility testing of resistant subpopulations after exposure showed identical double zone of inhibition, suggesting it was not caused by induction.

Conclusions: VITEK 2 automated system can exhibit very major error for both A. baumannii and E. coli harbouring armA. Considering widespread dissemination of armA, further study is needed to investigate the mechanism and frequency of this phenomenon in 16S rRNA methylase-producing Gram-negative bacteria.

P814 Disc diffusion test results after 6 hours in major pathogens from blood cultures

M. Sundqvist*, S. Bengtsson, R. Smyth, G. Kahlmeter (Växjö, SE)

Objectives: Standard empirical therapy of severe bacterial infections usually includes a β-lactam antibiotic. The increasing resistance to these agents has forced many countries to make changes in the empirical treatment schedules often resulting in less efficient treatment. In an area with relatively low frequency of septicaemia caused by β-lactam-resistant bacteria rapid and reliable phenotypic susceptibility testing would be of great importance to avoid inappropriate and/or less effective treatment regimens.

Methods: A susceptibility test based on disc diffusion on Mueller-Hinton agar supplemented with 5% horse blood and 20 mg/L of NAD and incubation at 35°C in CO2 was developed and wild-type distributions for major β-lactam drugs determined. Zone diameters were measured after 6h.

Two reference strains (ATCC 25922 and ATCC 29213) and sets of wildtype S. aureus (n=33) and E. coli (n=17) and well characterised isolates of MRSA (n=20) and ESBL-producing E. coli (n=20) were used to define wild-type and non-wild type zone diameter distributions for a limited set of β-lactam drugs. Inocula corresponding to viable counts of type strains in BacT/Alert (bioMérieux) blood culture bottles following 0–20h post signal for positivity were investigated.
Results: Reference strains and all clinical isolates grew sufficiently after 6h. Zone diameters were reproducible (to within ± 2 mm for the blood culture bottle inocula (0.4–0.6 McF)). The method could distinguish between micro-organisms without and with resistance mechanisms, e.g. methicillin-resistance using cefoxitin (10 μg) and ESBL-detection using both cefotaxime (5 μg) and ceftazidime (10 μg).

Conclusion: The described phenotypic method for rapid susceptibility testing can exclude the presence of important β-lactam resistance mechanisms in S. aureus and E. coli after 6 hours incubation using direct inoculation from blood culture bottles. This prevents the use of suboptimal therapy in patients with severe infections and a premature switch to empirical therapy with "rescue" drugs.
**P818** Impact of different EUCAST and CLSI interpretive breakpoints on antimicrobial susceptibility of *Pseudomonas aeruginosa* – SMART 2007

R. Bada†, S. Bouchillon, S. Hawser, D. Hoban, A. Johnson, M. Hackel, J. Johnson (Schaumburg, US)

**Objectives:** CLSI guidelines are used in many countries; however, in Europe EUCAST breakpoints (BPs) are primarily used. Since EUCAST BPs often differ from CLSI’s, we evaluated the impact on reported susceptibility of *P. aeruginosa* using EUCAST vs. CLSI BPs for selected anti-pseudomonal agents.

**Methods:** Investigators in 93 hospitals in 33 countries in Europe, North and South America, Asia/Pacific, Middle East, and Africa collected up to 100 consecutive Gram-negative isolates from intra-abdominal infections in 2007; 654 of the organisms were *P. aeruginosa*. Minimum inhibitory concentrations (MICs) were determined by broth microdilution following CLSI methods, and results for drugs commonly used to treat *P. aeruginosa* were interpreted using both CLSI and EUCAST BPs.

**Results:** see the table.

<table>
<thead>
<tr>
<th>Drug</th>
<th>CLSI BPs</th>
<th>EUCAST BPs</th>
<th>% S</th>
<th>% I</th>
<th>% R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>16/32/64</td>
<td>8/16/32</td>
<td>87/4/9</td>
<td>74/12/14</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>8/16/32</td>
<td>8/16/32</td>
<td>75/10/15</td>
<td>75/25/10</td>
<td></td>
</tr>
<tr>
<td>Cefazolin</td>
<td>8/16/32</td>
<td>8/16/32</td>
<td>74/6/20</td>
<td>74/26/1</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1/2/4</td>
<td>0.5/1/2</td>
<td>72/24/4</td>
<td>69/3/28</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>4/8/16</td>
<td>4/8/16</td>
<td>73/8/19</td>
<td>73/8/19</td>
<td></td>
</tr>
<tr>
<td>Levofloxin</td>
<td>2/4/8</td>
<td>1/2/4</td>
<td>72/4/24</td>
<td>65/6/29</td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>64/128</td>
<td>16/128</td>
<td>85/15</td>
<td>76/24</td>
<td></td>
</tr>
</tbody>
</table>

S = susceptible, I = intermediate, R = resistant.

**Conclusions:**
- The tendency of EUCAST BPs to be one doubling dilution lower than CLSI’s caused reductions in %S with 4 drugs (amikacin, ciprofloxacin, levofloxacin, and pip-tazo), the other 3 drugs evaluated (cefepime, ceftazidime, and imipenem) had equivalent %S since the S BP was the same in both guidelines.
- The largest decreases in %S were seen with amikacin and piperacillin-tazobactam: 13 and 9%, respectively.
- Absence of an “I” category in EUCAST for 3 drugs (amikacin, cefepime, and pip-tazo) would result in reported resistance rates to be 6–10% higher than in countries using CLSI BPs.
- Using EUCAST guidelines, none of the study drugs achieved %S higher than 76%; even using CLSI only 2 drugs (amikacin and pip-tazo) were above 80%. If 90% or even 80% susceptible is considered to be a minimum indication of a drug’s utility for empiric therapy, the list of agents remaining active in vitro against *P. aeruginosa* is limited.

**P819** Computerised antibiotic susceptibility prediction: accounting for prior knowledge and cross-resistance

S. Andreassen*, A. Zaloumita, G. Kariv, L. Lebovici, M. Paul (Aalborg, DK; Petah Tiqua, IL)

**Objectives:** To develop an automated method for estimation of a broad range of antibiotic susceptibilities given a restricted antibioticogram.

**Methods:** Amended susceptibility for a non-tested antibiotic was determined using the probability of it being sensitive (S) to this antibiotic a priori; available susceptibility results for all antibiotics in the antibioticogram and cross-resistance, expressed as the conditional probability of resistance for non-tested antibiotics given the tested antibiotics. The method was derived using a bacteremia database of 3347 clinically significant isolates (CSI) collected between 2002–2004 and validated on a bacteremia database of 4848 CSI between 2006–2008, both at Rabin Medical Center, Israel. The Brier score (BS) was used to measure the accuracy of the predicted amended susceptibilities. This score measures the distance between the predicted susceptibility and the actual outcome (0% indicates perfect agreement and 100% complete deviation).

**Results:** The table exemplifies how the susceptibility of *Acinetobacter* to imipenem is amended. The knowledge that *Acinetobacter* is sensitive to amikacin and meropenem increases the odds for imipenem=S by odds ratios (OR) of 5.8 and 21.5, respectively. Resistance (R) to minocycline reduces the odds by an OR of 0.47. The remaining antibiotics carry insignificant ORs (p≥0.10). In the proposed method the biggest OR (21.5) is then multiplied by the smallest OR (0.47) and the a priori odds for imipenem=S (56/44 = 1.27) is multiplied by that product. The resulting amended probability for imipenem=S is 93%. Across all pathogen/antibiotic combinations in the derivation DB, the BS for a priori susceptibilities was 39%, reduced to 25% for amended susceptibilities. For the validation DB, the BS for a priori susceptibilities was 41%, reduced to 29% for amended susceptibilities, indicating that there is a significant advantage to the amendment (p < 0.001).

**Conclusion:** Amended susceptibilities can be used to guide the prescription of antibiotics not included in the antibiogram. This is necessary to allow for a restricted antibiotic panel testing in the laboratory, to assist clinicians and to improve the management of polymicrobial infections. The computerised algorithm will be incorporated into the TREAT computerised decision support system for antibiotic treatment.

<table>
<thead>
<tr>
<th>Drug</th>
<th>% a priori coverage</th>
<th>Susceptibility</th>
<th>Odds for S</th>
<th>Odds for R</th>
</tr>
</thead>
<tbody>
<tr>
<td>penbriti</td>
<td>3</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pipera</td>
<td>11</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kefin</td>
<td>2</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cefuroxi</td>
<td>1</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cefatura</td>
<td>17</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ceftruax</td>
<td>3</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>genta</td>
<td>25</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tobra</td>
<td>34</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>amikacin</td>
<td>30</td>
<td>S</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>chloram</td>
<td>17</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>septrin</td>
<td>14</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>oflo</td>
<td>15</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aztreona</td>
<td>3</td>
<td>R</td>
<td></td>
<td></td>
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<tr>
<td>colistin</td>
<td>98</td>
<td>S</td>
<td></td>
<td></td>
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<tr>
<td>tetra</td>
<td>10</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>augmenti</td>
<td>5</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cipro</td>
<td>15</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>minocycline</td>
<td>52</td>
<td>R</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>tazocin</td>
<td>14</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cefipram</td>
<td>18</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unasyn</td>
<td>87</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ertapenem</td>
<td>14</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>imipenem</td>
<td>56</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>meropenem</td>
<td>57</td>
<td>S</td>
<td>21.5</td>
<td></td>
</tr>
</tbody>
</table>

**P820** Assessment of Microscan<sup>®</sup> rapid ID-AST versus conventional panels for Gram-negative bacteria

M. Zoppelletto*, M. Gaino, A. Gardumi, P. Lanzafame, P. Ober (Trento, IT)

**Objectives:** The new rapid MicroScan<sup>®</sup> Synergies NEG BP Combo Panel 7 (SI+NBPC7) for Gram-negative bacteria was assessed for the ability in bacteria identification (ID) and antibiotic susceptibility testing (AST) compared to Microscan<sup>®</sup> conventional panel. The new system combines early MIC results with the capability of holding and reading panels incrementally for up to 18 hours.

**Methods:** The accuracy of the new system SI+NBPC7 was evaluated by comparing the performance of the new system to Microscan<sup>®</sup> NEG33 and NUC37 panels. A combination of 50 fresh and 29 stock Enterobacteriaceae and *Pseudomonas aeruginosa* isolates was tested, comprising ESBLs, β-lactamases AmpC-like and carbapenemases producers.
Results: For the organisms tested, rapid results were available from 4.5 to 18 hours, with 75% of the results final at 8 hours and more than 90% of the ID complete by 2.5 hours. Performance results indicated that the essential agreement (major and minor errors) between the test system and the reference method was greater than 95% for all the antibiotics tested. 18 (1.03%) very major errors were noted in the performance of these drugs: ampicillin (3/79), trimethoprim/sulfa methoxazole (3/79), levofloxacin (2/79), ciprofloxacin (2/79), gentamicin (1/79), cefuroxime (2/79), imipenem (1/79), meropenem (1/79), tobramycin (2/79), amikacin (1/79), 44 (2.53%) minor errors in AST was observed: piperacillin/tazobactam (4/79), cefepime (4/79), gentamicin (3/79), tobramycin (7/79), amoxicillin/K clavulanate (4/79), ceftazidime (7/79), piperacillin/tazobactam (1/79), cefotaxime (1/79), imipenem (1/79), levofloxacin (1/79), cefazolin (6/79), ciprofloxacin (1/79), ampicillin (1/79), nitrofurantoin (1/79). The errors in ID was 10/79 (12.65%).

Conclusions: This study showed that the new MicroScan® rapid panel SI-NBPC7 for Gram-negative bacteria provides rapid, accurate results for ID and against all antimicrobial agents when compared to conventional panels. The new panels are very useful to decrease time of microbiology tests keeping high quality levels.

P821 Use of a chromogenic media to detect enteric carriage of multidrug-resistant Acinetobacter baumannii in critically ill patients
N. Gordon*, D. Wareham (London, UK)

Objectives: Multi-drug resistant A. baumannii (MDRAB) has emerged as an important nosocomial pathogen in the critically ill. The organism is difficult to eradicate from the environment and many centres have experienced ongoing outbreaks despite heightened infection control procedures. Selective decontamination of the gut (SDD) has been used as a useful technique that provides same day susceptibility results (18−24h)

Methods: 48 stool samples and 23 perineal swabs obtained from ICU patients were plated on to Chromagar (CAAB) and then inoculated into peptone broths. The agar plates were examined after 18 hours, with 75% of the results final at 8 hours and more than 90% of SDD might be a sensible approach for the prevention of A. baumannii infection we evaluated a novel chromogenic medium for the detection of MDRAB in enteric samples from patients in intensive care (ITU).

Results: 34 samples were culture positive for MDRAB compared with 32 positive by PCR. 27 patients were known to be colonised at the time of sampling but in 7 individuals the organism had not been found in previous cultures of clinical specimens. There was a significant correlation between colonisation elsewhere and stool carriage (p < 0.05).

Conclusions: CAAB appears to be a useful media for screening for the carriage of MDRAB. It compares favourably with molecular methods and is able to isolate common epidemic clones. The study also confirms the human gut as an important reservoir of MDRAB in critically ill patients and raises the possibility of SDD as an additional measure in outbreak management.

P822 Evaluation of direct E-test on lower respiratory tract samples using a chromogenic agar medium: a rapid procedure for antimicrobial susceptibility testing
E. Cercenado*, M. Martin, R. Insu, P. Martin-Rubial, M. Rivero, E. Bouza (Madrid, ES)

Objectives: We have previously demonstrated the accuracy of direct Etest (DET) on lower respiratory tract (LRT) samples from ICU patients as a rapid procedure for antimicrobial susceptibility testing (Cercenado et al., Diagn. Microbiol. Infect. Dis. 2007; 58:211) which may be crucial for modifying therapeutic regimens. In this study we evaluate a modification of this technique using this chromogenic agar medium in order to generate rapid susceptibility results and organism identification.

Methods: Over a period of 6 months we received 272 LRT samples from ICU patients. Samples were processed by DET onto chromogenic Mueller-Hinton agar (IZASA, Spain) as well as by the standard quantitative culture followed by identification and susceptibility testing by microbroth dilution method (MBD). Oxacillin, piperacillin/tazobactam, cefepime, imipenem, ciprofloxacin, and amikacin were the antimicrobials evaluated.

Results: A total of 143 LRT samples (94 monomicrobial and 49 polymicrobial) yielded significant counts in the MBD with microorganisms able to grow on chromogenic agar (Haemophilus spp., S. pneumoniae and M. catarrhalis were excluded from the analysis). Microorganisms isolated (n = 192) were: S. aureus (54), P. aeruginosa (44), A. baumannii (24), S. maltophilia (15), E. coli (14), Klebsiella spp. (14), P. mirabilis (11), and other Enterobacteriaceae (16). Overall, 92.7% of the isolates were recovered by the DET-chromogenic at 18h, and 100% at 24h (12 S. maltophilia isolates). Among the 731 microorganism-antibiotic combinations evaluated, there was a total agreement with the MBD in 94.9%. There were 5 very major errors (0.68%) (all in polymicrobial cultures), 29 major (3.9%) (9 with imipenem and A. baumannii), and 4 minor (0.5%). Discrepancies corresponded to 20 monomicrobial and 18 polymicrobial cultures, and the majority occurred with imipenem (14.4%) and cefepime (5.6%). The chromogenic medium allowed identification by colours and facilitated readings especially in polymicrobial cultures.

Conclusions: DET on respiratory samples is a reliable and clinically useful technique that provides same day susceptibility results (18−24h) comparable to those obtained by MBD. The use of chromogenic agar medium constitutes an improvement that facilitates readings and allows concomitant identification of the pathogen involved.

P823 Evaluation of VITEK 2 for identification of enterococci and detection of vancomycin resistance
H. Adler*, S. Ozcan, S. Sax, R. Frei (Basel, CH)

Objective: To evaluate the VITEK 2 (bioMerieux, Marcy l’Etoile, France) for the identification of Enterococcus faecalis, E. faecium, E. gallinarum and E. casseliflavus and for the detection of vancomycin resistance.

Methods: We examined a total of 83 enterococcal isolates. Isolates comprised 26 E. faecium and 24 E. faecalis, 26 of them with acquired vancomycin resistance (VanA, VanB). Additionally, 33 isolates with natural vancomycin resistance (22 E. gallinarum, 11 E. casseliflavus) were tested. Isolates were identified with the VITEK 2 colorimetric GP card. For susceptibility testing the antimicrobial susceptibility testing card P 534 was used. MICs were interpreted using the breakpoints recommended by the CLSI. Identification and vancomycin resistance were molecularly confirmed with the GenoType Enterococcus test (Hain Lifescience, Nehren, Germany) as gold standard.

Results: All isolates of E. faecalis and E. faecium were correctly identified as were 20 of 22 isolates of E. gallinarum and 7 of 11 isolates of E. casseliflavus. In 6 isolates Vitek 2 could not differentiate between E. gallinarum and E. casseliflavus. Vitek 2 results of susceptibility testing are presented in table 1. Vancomycin resistance was detected in all isolates carrying a vanA or vanH gene, thus the test was 100% sensitive for acquired vancomycin resistance. Teicoplanin resistance was missed in one isolate of VanA, consequently the isolate resembled a VanB phenotype. Sensitivity for natural vancomycin resistance (VanC) was 91%. Of the isolates with VanC, 48% had a MIC of ≥32 mg/l (resistant), thus resembling a VanB phenotype.

Conclusions: VITEK 2 is an excellent tool for the identification of E. faecalis and E. faecium (100% correct identifications). The system was less able to differentiate between E. gallinarum and E. casseliflavus (82% correct identification, 18% low discrimination). However, since
both species have natural resistance to vancomycin, the clinical significance of separating them is minimal. VITEK 2 is a useful means for the detection of vancomycin resistance in enterococci. However, both identification and susceptibility testing should be performed in order not to miss natural vancomycin resistance and to discriminate between natural and acquired vancomycin resistance.

Table 1. Susceptibilities as determined by Vitek2

<table>
<thead>
<tr>
<th>Organism (no.)</th>
<th>Vancomycin</th>
<th>Teicoplanin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>vanA VRE (12)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>vanB VRE (13)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>vanA+vanB VRE (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>vanC1 VRE (22)</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>vanC2 VRE (11)</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>VSE (24)</td>
<td>24</td>
<td>0</td>
</tr>
</tbody>
</table>

S sensitive, I intermediate, R resistant.

VRE vancomycin resistant Enterococcus, VSE vancomycin susceptible Enterococcus.

**P824**

Comparison of the impact of direct plating versus a short or overnight pre-enrichment on detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens


**Objectives:** Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from screening cultures is crucial for effective infection control. While an overnight pre-enrichment (On-En) can increase chances of MRSA detection, time to result is 48 hrs. A short, 4-hour pre-enrichment (Short-En) would enable next day results, however its advantage over direct plating (DP) is not known. We compared the impact of DP to plating after Short-En or On-En on MRSA detection from screening samples.

**Methods:** Fifty two nasal and groin swabs from 25 patients previously identified as MRSA carriers were collected in BHI + glycerol. 10 μl sample was spiral plated directly on a chromogenic medium, CHROMagar MRSA (BD, Belgium) or on mannitol-salt agar with 4 μg/ml cefoxitin (MSAC), or added to enrichment broth (Tryptone soya broth with 2.5% salt, 20 μg/ml aztreonam, and 3.5 μg/ml cefoxitin). 10 μl of enrichment broth was spiral plated on CHROMagar and on MSAC after a Short-En and On-En. Colony counts were done for agar cultures after overnight incubation, and putative MRSA colonies confirmed by standard tests. Non-parametric comparisons were made using Friedman’s test. Differences in readings (MRSA positive/negative) at the three time-points were modelled using a logistic approach. Generalised estimating equation was used to account for repeated measures over time and the Score test to assess differences between the three time-points.

**Results:** Of the 52 samples, 9 were negative for MRSA at all three time-points. Plate readings for MRSA positivity after DP or Short-En did not differ significantly (P=0.317), and showed clear differences after ON-En in comparison to DP or Short-En (P=0.002 and 0.004, respectively). Two MRSA negative samples gave positive results after ON-En (4% misclassification error). Colony counts differed significantly between DP (mean CFUs/ml: 3.91×10⁷, 95% CI: ±9.66×10⁶), Short-En (mean CFUs/ml: 6.79×10⁴, 95% CI: ±1.22×10³), and ON-En (mean CFUs/ml: 2.31×10⁶, 95% CI: ±1.16×10⁴) (Friedman’s chi-square = 53.91, degrees of freedom = 2, P=1.964e-12) (Figure). Of the 52 samples, 60% (n=30) showed similar colony counts after DP and Short-En, 23% after DP and ON-En, and 31% after Short-En and ON-En.

**Conclusions:** A Short-En does not offer a significant increase in MRSA detection in comparison to DP and cannot replace an overnight enrichment at least when culture-based methods are used for downstream processing.

![Figure: Colony count profiles and trend after DP, Short-En, and ON-En.](Image)

**P825**

Performance of Oxoid Brilliance MRSA, a new chromogenic medium

E.J.M. Verkade*, S. Elberts, C.J.M.M. Verhalst, J.A.J.W. Kluytmans (Breda, NL)

**Objectives:** To assess the in vitro sensitivity and specificity of Oxoid Brilliance MRSA for the detection of MRSA.

**Methods:** A collection consisting of 235 methicillin-resistant *Staphylococcus aureus* (MRSA) strains, 284 methicillin-susceptible *Staphylococcus aureus* (MSSA) strains, and 265 coagulase-negative staphylococci (CNS) was used. Identification of strains as *S. aureus* and as being methicillin resistant had been performed by duplex PCR for the mecA gene and the coagulase gene. Strains were selected on the basis of their different phage types. The isolates were inoculated onto agar plates to obtain fresh growth. From the resulting cultures, a suspension with a 0.5 McFarland standard was made, and subsequently, 10 microliter was streaked onto an Oxoid Brilliance MRSA plate. The plates were read after 20 hours of incubation at 35°C. Growth of colonies showing blue coloration were considered to be positive (indicating MRSA). No growth or colonies with colours other than blue were considered negative. The procedure was performed as recommended by the manufacturer.

**Results:** Twenty-nine MRSA strains gave discordant results, and a PCR for the mecA gene was performed on these isolates. A total of 28 (97%) of the MRSA strains had a negative result with the mecA PCR. These strains were removed from the analysis, according to the protocol of the study. The results obtained with Oxoid Brilliance MRSA are shown in Table 1. The sensitivity was 99.6% and the specificity was 97.4%.

**Conclusion:** Oxoid Brilliance MRSA can detect a large number of different MRSA strains and is a sensitive and specific tool for differentiation between CNS/MSSA and MRSA in vitro.

Table 1. Results for Oxoid Brilliance MRSA medium after 20 hours of incubation

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of strains with a positive test result/total no. of strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA</td>
<td>5/284 (1.8)</td>
</tr>
<tr>
<td>CoNS</td>
<td>9/265 (3.4)</td>
</tr>
<tr>
<td>Total (CoNS + MSSA)</td>
<td>14/549 (2.6)</td>
</tr>
<tr>
<td>MRSA</td>
<td>234/235 (99.6)</td>
</tr>
</tbody>
</table>
Antimicrobial susceptibility testing and resistance detection

**P826** Performance of three methods for determining the susceptibility to daptomycin and three other antimicrobials of Enterococcus spp.

F. López-Fabal*, A. Barillo, Y. Gil, J.L. Gómez-Garcés (Madrid, ES)

**Objectives:** To compare the E-test, an automated test (Wider) and broth microdilution (as the reference test) used to determine the susceptibility of clinical isolates of Enterococcus spp. to vancomycin, teicoplanin, linezolid and daptomycin.

**Methods:** Microorganisms were isolated from clinical specimens obtained from patients in three hospitals in south Madrid. 100 strains identified in 2007 using standard procedures were included. For quality control, we used Enterococcus faecalis ATCC 29212. The broth microdilution method conducted according to CLSI guidelines was used as the reference method. For daptomycin, the medium was enriched with calcium cations to achieve a final concentration of 50 mg/L.

**Results:** All isolates were tested using the three methods and results compared to those obtained using the broth microdilution method as reference. The MICs of the control strains were within their known ranges. The table shows the results obtained using the two methods compared to the reference method.

<table>
<thead>
<tr>
<th>Organism group</th>
<th>N</th>
<th>Drug</th>
<th>Agar Dil. %S</th>
<th>Etest %S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerococcus spp.</td>
<td>45</td>
<td>P</td>
<td>68.2</td>
<td>90.2</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>430</td>
<td>CFX</td>
<td>85.5</td>
<td>57.8</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>239</td>
<td>MER</td>
<td>100</td>
<td>89.5</td>
</tr>
</tbody>
</table>

**Conclusions:** The Epsilon test (Etest) is a simple method to determine minimum inhibitory concentrations (MICs). The aim of the present method as the reference. The MICs of the control strain were within their known ranges. All the Staphylococcus spp. scored as susceptible to the four antibiotics. The table shows the results obtained using the other two methods compared to the reference method.

**Conclusions:** Our findings indicate that both the E-test and Wider tests are reliable methods for routine microbiology laboratory use to determine the antimicrobial susceptibility of staphylococci isolated from clinical samples.

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**P827** Performance of three methods for determining the susceptibility to daptomycin and three other antimicrobials of methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase negative Staphylococcus spp.

F. López-Fabal, A. Barillo, Y. Gil, J.L. Gómez-Garcés* (Madrid, ES)

**Objectives:** To compare the E-test, an automated test (Wider) and broth microdilution (as the reference test), to determine the susceptibility to vancomycin, teicoplanin, linezolid and daptomycin of clinical isolates of methicillin resistant Staphylococcus aureus (MRSA) and methicillin resistant coagulase negative Staphylococcus spp. (MRCNS).

**Methods:** The microorganisms included were isolated from clinical specimens from patients of three hospitals in south Madrid. Selection was based on their resistance to methicillin. 60 MRSA and 60 MRCNS strains identified in 2007 using standard procedures were included. As a quality control we used S. aureus ATCC 29213. As the reference method we conducted the broth microdilution method according to CLSI guidelines. For daptomycin the culture medium was enriched with calcium cations to achieve a final concentration of 50 mg/L. Isolates were classified as susceptible, intermediate or resistant according to CLSI criteria for each antimicrobial and microorganism.

**Results:** All isolates were tested using the methods described above and the results compared to those obtained using the broth microdilution method. The table shows the results obtained using the two methods compared to the reference method.

**Conclusions:** Our findings indicate that both the E-test and Wider tests are reliable methods for routine microbiology laboratory use to determine the antimicrobial susceptibility of staphylococci isolated from clinical samples.
work was to assess the suitability of Clinical Laboratory Standards Institute (CLSI) broth microdilution (BMD) breakpoints to categorise MICs obtained by Etest.

**Method:** The strains were ATCC 33560 and 59 Campylobacter sp clinical isolates selected among the 331 obtained from stools of patients with diarrhoea between February 2007 and September 2008. Categorisation of clinical isolates was accomplished through disk diffusion; CLSI (M-45, 2006) guidelines were followed; plates were incubated 24 hours at 42°C. Etest was performed according to manufacturer instructions; clinical isolates were incubated 24 hours at 42°C; all were tested twice. Every reading was performed by the same observer. ATCC 33560 was evaluated 25 times; two plates were prepared daily, one of them was incubated 48 hours at 37°C and the other 24 hours at 42°C.

**Results:** According to disk diffusion categorisation 55 clinical isolates were susceptible to erythromycin and 24 to ciprofloxacin. Strains with erythromycin inhibition diameters below 6 mm had MICs >256 mg/l while those with zones broader than 6 mm had MICs ≤ 4 mg/l; there were not strains with MICs between 8 and 256 mg/l. Ciprofloxacin resistant isolates according to CLSI disk diffusion criteria had MICs >32 mg/l, and MICs of susceptible ones were ≤ 0.5 mg/l; we did not find strains with MICs between 1 and 32 mg/l. Ten ciprofloxacin and 22 erythromycin MICs interseries discrepancies were found; none of them entailed category changes according to CLSI BMD susceptibility criteria. When CLSI BMD breakpoints were employed to categorise MICs obtained by Etest, agreement with disk diffusion was complete. Etest incubated at 37°C yielded two ATCC erythromycin MIC values one dilution over the upper limit established by CLSI for BMD and this was the case with nine ciprofloxacin and three erythromycin MICs when plates were incubated at 42°C.

**Conclusion:** Our data indicate that CLSI interpretive criteria for BMD could be tentatively applicable to MICs obtained by Etest. Incubation at 37°C for 48 hours seems to provide more accurate results. Correlation between Etest and CLSI BMD standard method must be established to confirm our results; studies including a greater number of erythromycin resistant strains would be of special interest.

**P830** Molecular genetic analysis of 23S RNA mutations associated with clarithromycin resistance in *Helicobacter pylori* strains isolated in St. Petersburg, Russia

E. A. Tarasova*, M. A. Suvorova, A. V. Scarratt, R. S. Ferman, A. B. Zhebrun, A. N. Suvorov (St. Petersburg, RU)

**Objectives:** Resistance to antibiotics quite often interferes with effective eradication of Helicobacter pylori. It is known that resistance to clarithromycin which interferes with ribosomal synthesis can be achieved by point mutations in 23S ribosomal RNA. Most of the claR mutations described in the scientific literature are the transitions of adenine to guanine in the positions A2142G or A2143G. Recently other mutations of such kind have been described (Garrido L, 2007; Toracchio S, 2004; Umegaki N, 2000). After the analysis of *H. pylori* clinical strains collection isolated in St. Petersburg we have determined that most common claR mutations were A2142G, A2143G and T2717C. All these mutations are possible to determine by PCR with the following restriction analysis. However, in some cases of claR strains those mutations were not found.

The aim of present work was to analyze the structure of mutations in 23S RNA in *H. pylori* strains isolated in St. Petersburg, Russia.

**Materials and Methods:** 20 patients with chronic gastritis associated with *H. pylori* infection were selected. Clarithromycin resistance of the strains was tested by the disk diffusion method. Region corresponding to 23S RNA was amplified by PCR, digested with MboII, Bsal and Hhal and subjected to DNA sequencing.

**Results:** The presence of *H. pylori* among the group of patients under study was determined by urease test and by PCR employing the primers corresponding to several *H. pylori* genes (UreC, UreI, CagA). 7 strains under study were found to be resistant to clarithromycin and 4 out of 7 were resistant to digestion with MboII, Bsal and Hhal, which suggested the absence of mutations A2142G, A2143G and T2717C. After DNA sequencing it was determined that the rest of the strains carried different claR mutations: T2182C, C2195T or C2288T. Mutation T2182C have been described previously (Khan R, 2004; Kim, 2002; Posteroar R, 2006; Chilia L, 2005) but mutations C2195T and C2288T were found only together with A2142G or A2143G (Posteroar P, 2006; Pimbara E, 2007).

**Conclusion:** Molecular study of the structure of clarithromycin resistant 23S RNA mutations in St. Petersburg Russia allowed determining the broad spectrum of mutations some of which were determined for the first time. This fact leads to conclusion that commonly used for such diagnostics PCR with the following restriction analysis might be not appropriate for determining clarithromycin resistant *H. pylori* strains in Russia.

**P831** Use of peptide nucleic acid probes as a new method for the detection of clarithromycin resistance in *Helicobacter pylori* strains

L. Cerqueira*, N. F. Azevedo, N. Guimarães, C. Figueiredo, C. W. Keevil, M. J. Vieira (Braga, Porto, PT; Southampton, UK)

**Objectives:** The treatment of patients infected with *H. pylori* is being seriously compromised due to increased antibiotic resistance patterns (e.g. amoxicillin, clarithromycin and metronidazole). For that reason, the purpose of this study was to develop a fast and more efficient method to test clarithromycin resistance in clinical samples using fluorescence in situ hybridisation (FISH).

**Methods:** There are several phenotypic, cultivation-dependent methods able to identify the clarithromycin resistance in *H. pylori* strains, but they are all fastidious and growth is time-consuming. Three mutations in the 23S rRNA of *H. pylori* are strongly associated with clarithromycin resistance. In these mutations, an adenine is replaced by a guanine at positions 2142 and 2143, or by a cytosine at position 2142. Hence, we developed a set of peptide nucleic acid (PNA) probes for the identification of target sequences for the different clarithromycin resistance polymorphisms. PNA molecules are DNA synthetic mimics with a non-charged backbone, due to their chemical configuration. As such, they present a lack of electrostatic repulsion, resulting in improved thermal stability compared with DNA/DNA duplexes.

**Results:** After probe design, an optimisation of the hybridisation conditions, like temperature, pH, ionic strength and formamide concentrations, was performed. To ensure specificity and sensitivity, probes have been tested against resistant and susceptible strains of *H. pylori*.

**Conclusions:** This novel PNA FISH method will facilitate a more prompt (<3 h) diagnosis of *H. pylori* clarithromycin resistance in clinical samples such as gastric biopsies, thus allowing a more rational patient treatment.

**P832** Evaluation of the Vetek 2 YST and AST-YS01 card for the identification and antifungal susceptibility testing of *Candida* spp.

S. Nya*, S. Vijgen, K. Magerman, A. Boel, R. Cartuylets (Hasselt, Aalst, BE)

**Objectives:** The YST and AST-YS01 cards for Vetek 2 were evaluated for their accuracy and rapidity to identify yeasts and to perform susceptibility testing for fluconazole (FLU), voriconazole (VO) and amphotericin B (AMB) respectively.

**Methods:** A total of 69 yeast strains were tested (*Candida glabrata* n = 29, *Candida albicans* n = 22, other *Candida* species n = 18). All strains were clinical isolates from unrelated patients.

Each yeast strain was tested with Vetek 2 (bioMérieux), using the YST and AST-YS01 cards, according to the manufacturer's instruction.

**Results:** Results of identification and susceptibility testing with Vetek 2 YST and AST-YS01 cards were compared to results of previous biochemical identification, confirmed by ITS2 fragment length determination on CEQ8000 (Beckman Coulter), and susceptibility testing results with
micro-broth dilution according to CLSI guidelines. MIC results were categorised according to CLSI breakpoints (document M27-A3/S3).

All strains were also sent to the Belgium Reference Laboratory for confirmation of identification and susceptibility testing.

Results: Three strains (4%) were falsely identified with Vitek 2 YST: 1 C. albicans was misidentified as C. glabrata and 2 C. glabrata strains were misidentified as C. albicans and Rhodotorula glutinis respectively. Vitek 2 did not determine any MIC values for 2 C. glabrata strains (3%) due to an insufficient growth in the control well. For Rhodotorula glutinis the Vitek 2 expert system has no interpretation criteria. These 3 strains were excluded from further analysis.

MIC values for AMB and VO of all C. albicans isolates were comparable to the reference method. The MIC value of FLU obtained with the Vitek 2 was different for 2 strains (9%), leading to a minor error for 1 strain (4.5%).

For C. glabrata, there was an essential agreement of 56%, 78% and 89% for FLU, VO and AMB respectively, leading to 8 (30%) minor errors for FLU, 1 (4%) minor and 1 (4%) major error for VO. For other Candida species an essential agreement of 94% and 88% was found for VO and AMB respectively without any errors. For FLU this was 82%, with one (6%) very major error.

Conclusions: The Vitek 2 YST is a simple and fast method for a reliable identification of Candida species. Performance of the new Vitek 2 AST-YS01 card for susceptibility testing seems to be species dependent. For C. glabrata, the AST-YS01 is rather unreliable, especially for fluconazole. For other Candida species this method is fairly consistent for VO and AMB.

Evaluation of the VITEK® 2 system for the susceptibility testing of Candida species and Stephanoascus ciferii with caspofungin, micafungin and posaconazole


Objective: The VITEK 2 System provides rapid, automated identification and susceptibility testing of yeast isolates. With the increasing prevalence of antifungal resistance, the ability to quickly and easily perform susceptibility testing with available antifungals is becoming more important. The purpose of this study was to determine whether caspofungin (CAS), micafungin (MCF) and posaconazole (POS) could be incorporated into the current VITEK 2 system menu for automated susceptibility testing of yeasts.

Methods: Over 450 isolates were tested in VITEK 2UO cards containing varying concentrations of CAS, MCF and POS. All of these strains were either Candida species or Stephanoascus ciferii. All strains were tested with all three antifungals using both IUO cards and a reference method. The reference method for all three was broth microdilution, which was performed according to Clinical Laboratory Standards Institute (CLSI) standards. Growth data were collected from the VITEK 2 cards and compared to the reference results. Analyses were then developed using these data.

Development results: Overall category agreement (CA) for CAS was 98.9% (449/454). Overall essential agreement (EA) was 99.6% (452/454). Overall category agreement (CA) for MCF was 99.6% (452/454). Overall essential agreement (EA) was 98.7% (448/454). Overall category agreement (CA) for POS was 95.4% (433/454). Overall essential agreement (EA) was 94.9% (431/454).

Conclusion: These development data indicate that the VITEK 2 can accurately detect resistance to CAS, MCF and POS in Candida species and in Stephanoascus ciferii.

Resistance to trimethoprim-sulfamethoxazole and Tropheryma whipplei

F.Fenollar*, J.M. Rolain, L. Abric, T. Papo, M.P. Chauweheid, D. Van de Beek, D. Rasolt (Marseille, Toulouse, Paris, FR, Amsterdam, NL)

Objectives: Whipple's disease (WD) is a chronic infection caused by Tropheryma whipplei and was fatal before the advent of antibiotics. A one-year treatment of oral trimethoprim-sulfamethoxazole is commonly used. The recent advances in culture of T. whipplei has allowed for full genome sequencing and antibiotic susceptibility testing, which has demonstrated resistance of T. whipplei to trimethoprim. Several mutations in the folP gene that encodes dihydropteroate synthase, the target of sulfonamides, has been reported by our team for one patient with clinically acquired resistance to trimethoprim-sulfamethoxazole, whereas no mutations were observed in 19 strains from patients without any evidence of clinical failure or relapse. Herein, we confirm, complete these data and propose a strategy in order to improve the management of WD.

Methods: Three new patients who experienced clinically acquired resistance to trimethoprim-sulfamethoxazole during treatment were reported as well as one patient with biological failure. Sixty-two folP sequences from DNA samples of 59 WD patients were also obtained.

Primers were designed according to the two available complete genomes of T. whipplei to frame the 801-bp folP. The nucleotide and amino acid sequences obtained were compared using the CLUSTALW program.

Results: The three new patients who experienced clinically acquired resistance to trimethoprim-sulfamethoxazole during treatment, were all verified by positive PCR analysis. The patient with biological failure only showed positive PCR. From the sixty-two sequences, eight different amino acid sequence types were found. Among the detected amino acid changes, two positions (N4S and S232F) significantly predicted secondary failure (in four out of five cases). The sensitivity of the N4S substitution to predict resistance before treatment was 50%, with 98% specificity, a positive predictive value (PPV) of 75%, and a negative predictive value (NPV) of 94.4%. The sensitivity of the S234F change to predict failure before treatment was 66.7%, with a specificity of 96.4%, PPV of 50%, and a NPV of 98.2%. Besides, new mutations appeared in two out of three amino acid sequences with previous N4S or S234F changes.

Conclusion: We suggest that these mutations (N4S and S232F) should be detected at the time of the WD diagnosis by sequencing folP in order to avoid sulfamethoxazole monotherapy.

Epidemiology of VRE

Evaluation of the VITEK® 2 system for the susceptibility testing of Candida species and Stephanoascus ciferii with caspofungin, micafungin and posaconazole

A. Tedim Pedrosa*, A.R. Freitas, P. Ruiz-Garbajosa, F. Baquero, T.M. Coque (Madrid, ES; Porto, PT)


A. Tedim Pedrosa*, A.R. Freitas, P. Ruiz-Garbajosa, F. Baquero, T.M. Coque (Madrid, ES; Porto, PT)

Objectives: Ampicillin-resistant Enterococcus faecium (AREfm), which are mainly associated with CC17, has increasingly been reported in European hospitals in the last decade. They have been suggested as the substrate for vancomycin resistance in this species although little is known about their content on mobile genetic elements. We analyzed the plasmid diversity of AREfm and ampicillin-susceptible (ASEfm) isolates in an area with a high rate of AREfm but low rate of vancomycin-resistant (VR) Efm.

Methods: We studied 72 Efm (52 AREfm and 20 ASEfm) causing bacteraemia (1995–2008). Antibiotic susceptibility was determined by CLSI microdilution. Clonality was established by PFGE-Smal and MLST. PPlasmid characterisation included determination of size and content (S1-PFGE), and identification of 27 relaxases (rel), 13 rep initiation proteins (rep) and 5 toxin-antitoxins systems (TA) by PCR and sequencing.

Results: AREfm isolates were classified in 30 PFGE-types and 10 STs belonging to CC17 (ST18 was predominant; 64%) and CC9 (2%). AREfm isolates were clonally heterogeneous, being identified as 11 STs clustering into CC1, CC9, CC17, CC22 and CC94. A great diversity of plasmid size (25–440 kb) and content (1–4/cell) being megaplasmids higher than 200 kb predominant. AREfm/ASEfm isolates harboured plasmids containing relaxases from pEF1 (87%/65%), pCIZ2 (83%/20%), pAD1 (25%/20%) or pRUM (14%/0%); rep proteins from
Dominance of ampicillin-resistant vancomycin-susceptible Enterococcus faecium-CC17 causing bacteremia over a 14-year period in a university hospital, Spain

P. Ruiz-Garbajosa*, L. Derdoy, E. Loca, F. Baquero, R. Cantón, T.M. Coque (Madrid, ES; Buenos Aires, AR)

Background: Bacteremia caused by ampicillin-resistant E. faecium (AREfm) has been increasingly reported in European hospitals due to the expansion of Clonal Complex-CC17. This was firstly observed in USA, preceding the emergence of vancomycin-resistant E. faecium (VREfm). CC17 is associated with ampicillin resistance and the presence of virulence/epidemiology markers esp and hyl and is enrichment in IS elements, specially IS16. The aim of this study was to analyze the population structure of Efm causing bacteremia in an area with a high rate of AREfm but low rate of VREfm during 14 years (1995–2008) and to investigate the presence of genetic determinants associated with CC17 epidemicity.

Methods: 167 Efm isolates (124 AREfm and 43 ASEfm) (167 patients) recovered from blood cultures (1995–2008) were studied. Susceptibility was determined by CLSI microdilution. Clonal relatedness was established by PFGE-SmaI and one representative isolate from each PFGE type was analyzed by MLST. esp and hyl genes, IS16, were detected by PCR.

Results: Ampicillin resistance among E. faecium isolates increased from 42% in 1995 to 100% in 2008. A low rate of VREfm (3.6%; 6/167) among bacteremic isolates was observed during all the studied period, with no trend to increase. By PFGE, 64 (21 AREfm, 43 ASEfm) types were identified. By MLST AREfm and ASEfm isolates were grouped into 11 STs and 40 STs respectively. Among AREfm, ST18 (62/124; 50%), ST17 (17/124; 14%) and ST16 (17/124; 14%) appeared as endemic and persistent STs during 14 years, whereas ST203 emerged more recently (2006) compromising 40% (16/41) of AREfm isolates recovering between 2006–2008. esp and hyl determinants were detected in 56% (69/124) and 45% (56/124) of AREfm isolates respectively. Within specific STs, ST16 showed the highest rate of isolates harbouring esp (94%) and hyl (82%) genes. None of the ASEfm isolates amplified esp and hyl genes. Presence of IS16 was investigated in a subset of isolates (AREfm, n = 92 and ASEfm, n = 20). IS16 was detected in 91% of AREfm population whereas was absent among ASEfm isolates.

Conclusions: An increased prevalence of AREfm isolates causing bacteremia along 14 years associated with a dominance and persistence of related CC17 STs. These STs, enriched in IS16 element and esp and hyl virulence/epidemiology markers that might contribute to hospital adaptation when compared with non-CC17 STs, might supply a substrate for the emergence of vancomycin resistance in our area.
Epidemiology of VRE

Isolation of glycopeptides-resistant enterococci from stool and blood samples of hospitalised patients during a three-year period


Objectives: The isolation of glycopeptide resistant enterococci from stool and blood cultures of hospitalised patients and their antibiotic resistance in a tertiary hospital in Greece during three years period (27/11/05–30/09/08).

Methods: During the study period 5047 stool samples were examined in our laboratory for GRE carriage and 15232 blood cultures for investigation of bacteraeemia. Enterococci were isolated on esculin azide agar with 6 μg/ml vancomycin and were identified by the automated system Vitek II (bioMerieux). The susceptibility was tested by disk diffusion agar method (Kirby-Bauer) and the MICs were determined by Vitek II system and E-test (AB, Biodisk, Solna).

Results: GRE strains were isolated in 63/5047 stool specimens (12.5%) and in 36/2483 patients with positive blood cultures (1.4%). Especially, 610 strains E. faecium (610/631, 97%) and 21 E. faecalis (21/631, 3%) were isolated from stool cultures and 31 strains E. faecium (31/36, 86%) and 5 E. faecalis (5/36, 14%) from blood cultures. The distribution per clinic of GRE strains from faeces was as followed: Haematology ward 177 strains (177/631, 28%), Intensive Care Unit (ICU) 53 (53/631, 8.4%), Nephrology ward 60 (60/631, 9.5%) Internal Medicine department 243 (243/631, 38.5%), Plastic-Burn Unit (PBU) 70 (70/631, 11%), Vascular Surgery department 2 (2/631, 0.3%) and Surgical department 25 (25/631, 4%). The distribution of GRE strains in the bacteraeemia cases was: Haematology ward 5, Gastroenterology ward 3, ICU 12, Internal Medicine department 6, PBU 9, Vascular Surgery department 1. Six cases of GRE bacteraeemia coexisted with GRE carriage; 2 from patients of ICU, 1 from Vascular Surgery department, 1 from Haematology ward, 1 from PBU and 1 from Gastroenterology ward. All isolated GRE strains from blood cultures and stool cultures were multi-drug resistant and sensitive to Linezolid.

Conclusions: The enteric carriage of GRE was approximately 13%. Most of the GRE strains were identified as E. faecium. Most of the strains, both from stool and blood cultures, were isolated in Haematology ward and Internal Medicine department. In six cases GRE bacteraeemia led to bacteraeemia. Antibiotic policy and strict enteric precautions should be implemented to restrict GRE carriage. As the risk of GRE bacteraeemia exists the epidemiological surveillance is necessary especially for immunocompromised patients.

Plasmid analysis of vancomycin-resistant Enterococcus faecium isolates from hospitals and aquatic environments in Portugal (1996–2008)

A.R. Freitas*, C. Novais, M.F. Francia, L. Peixe, T.M. Coque (Porto, PT; Santander, Madrid, ES)

Objectives: Vancomycin resistant (VR) enterococci constitute one of the most common nosocomial pathogens nowadays and they are mostly identified as CC17-E. faecium (Efm). VR among Efm isolates is frequently transferable by conjugation although little is known about the molecular epidemiology of their transferable plasmids. Plasmid diversity among VREfm from Portuguese hospitals was analysed in order to better understand the plasmid ecology and the dramatic recent spread of VRE in our country.

Methods: We analysed 75 VREfm vanA mostly belonging to CC17 (43 PFGE types; 18 Tn1546 types) from hospitalised patients in 6 hospitals of different cities (n=62) and from hospital waste waters and contaminated river samples (n=13) (1996–2008). Plasmid characterisation included determination of size and content, comparison of EcoRI/Clal-RFLP patterns, and identification of 27 relaxases (rel). 13 rep initiator proteins (rep) and 5 toxin-antitoxin systems (TA) by PCR, hybridisation and sequencing.

Results: A high diversity of plasmid content was observed (1–6/cell, 2–350 kb). All Tn1546 types, mostly containing iseI1 or isil216, were located on plasmids of variable size (30–250 kb; 95% conjugative). We identified 24 RFLP plasmid patterns with overrepresentation of 2 profiles corresponding to plasmids types of 60 kb and 90 kb, the most disseminated among hospitals and environment which were persistently recovered for long periods of time. All isolates harboured plasmids with similar content: relaxases from pEF1 (100%), pCI22 (80%) or pHTB (33%); rep proteins from prRUM (88%); pCI22 (65%); pRE25 (64%); IncI1 and pHTB (36% each); pEFPN1 (33%); Aae-Txe (10%) or omega-epision-zeta systems (14%). Relaxes from pAD1 (8%) and pRUM (4%), as well as rep proteins from pAD1 (7%) and pD11 (4%), were rarely detected. VanA-plasmids were predominantly derivatives of pEF1 plasmid containing rel-pEF1, mostly associated with rep-prRUM and eventually with rep from pRE25 and/or IncI1. pHTB-like plasmids which have been involved in the spread of VRE in Japan and USA, were rarely detected among 60 kb-plasmids and were not associated with Tn1546.

Conclusions: VREfm isolates recovered from Portuguese hospitals and aquatic environments contained plasmids with similar gene content over years, which indicate a high plasmid genetic stability among VRE in dissemination in the country and in the affected counties, respectively, are still unclear but under investigation. A national group has been formed to discuss strategies to prevent further spread of VRE and laboratory coordination of typing.
our area. The conjugal plasmids driving the spread of vancomycin resistance in our area are mosaics including mainly sequences from pEF1 and pRUM.

Impact of reduction of environmental and equipments positive cultures for VRE on the rates of infection due to VRE in an intensive care unit at a teaching hospital in Brazil

M. Persugini, S. Nomi, G. Lopes, I. van der Heijden, A. Mostachio, S. Costa* (Londrina, Sao Paulo, BR)

A variety of measures have been used to reduce VRE rates, the optimal approach however, is not well defined.

Objectives: The aim of this study was to evaluate the impact of a multidisciplinary process to monitor healthcare work (HCW) compliance with standard and contact precautions and the role of environment and equipments on the transmission of VRE.

Material: This study consisted of four periods baseline, pre, intervention and post-intervention period. Chi-square test was used to compare data pre and post intervention and Chi-square test for linear trend was used to evaluate the distribution of VRE and use of glycopeptides during the study period, the level of p < 0.05 was significant. PFGE was performed.

Results: E. faecium was the most frequent species isolated being responsible for 71% of positives cultures. Forty-six infection were documented, bloodstream infection 17 (47%) was the most frequent site. The educational intervention was given to 136 HCW. 706 opportunities were evaluated, the compliance with standard and contact precautions did not improve comparing pre and post-intervention period. However, the proportion of environmental and equipments positive cultures decreased significantly comparing pre (23.2%) and post-intervention (2.4%) period (p < 0.001) and was associated with decrease of VRE infection per 1,000 pts-day (p = 0.004). The use of vancomycin (DDD) did not change significantly over the study period (p = 0.97) and the use of teicoplanin increased (p < 0.001). Ninety percent of E. faecium belong to the same type.

Conclusions: In the present study, reduction of proportion of positive environmental and equipments cultures was associated with decreased of rates of VRE infections.

Vancomycin-resistant enterococci infection outbreak in the intensive care unit

D. Sponogiannis, M. Kalaitzopoulou, E. Kakasi, E. Mastrogiannidou, A. Vasiliadou, A. Papa, E. Kosmidou* (Thessaloniki, GR)

Objectives: Vancomycin Resistance Enterococcus Faecium (VREF) is becoming a significant pathogen in the Intensive Care Units (ICU) patients. In this study we present a VREF infection outbreak in the ICU of our hospital.

Methods: Two patients had documented both infection and colonisation. VRE was isolated in blood and stool culture of the first patient and in wound and stool culture of the second one. Screening of the rest of ICU patients by stool culture revealed one carrier. Phenotypic control and MIC's determination were performed by VITEK system (both microdilution panels). Additionally MIC's were confirmed with E-test according to CLSI standards. Multiplex PCR was used for the detection of rrs and ddl genes. Also special primers for VanA, VanB, VanC1, VanC2/C3 genes were used.

Results: Strains were identified as Enterococci faecium by both phenotypic and genetic methods. Resistance to vancomycin and teicoplanin was detected in all strains. MIC's determination by VITEK was ≥32 μg/ml for both glycopeptides. E-test revealed vancomycin's MIC > 256 μg/ml and teicoplanin's MIC=32 μg/ml in all strains. All strains were susceptible to linezolid. Molecular method (PCR) detected VanA gene in both patients and in the carrier too.

Conclusion: The results indicated potential survival of strains in the hospital environment and possible transmission among the hospitalised patients. The outbreak may be controlled by continuous implementation of an infection control program including improved hand-washing facilities.

Typing of vancomycin-resistant enterococci from Danish hospitals


Objectives: Vancomycin-resistant enterococci (VRE) are reported to increase in numbers in European hospitals. Vancomycin resistance can be encoded by seven different genes but only vanA and to a lesser extent vanB are widely prevalent among clinical isolates of enterococci. The E. faecium clonal complex 17 (CC17) has been associated with nosocomial outbreaks in five continents. The aim of the present study was to elucidate the molecular epidemiology of VRE in Denmark.

Methods: From January 2005 through October 2008, 61 vancomycin resistant enterococcal isolates causing invasive as well as non-invasive infections were referred by seven of the 15 Danish departments of clinical microbiology to Statens Serum Institut. All isolates were identified to species level by PCR, MICs of vancomycin were determined (Trek Diagnostic Systems, UK), and the presence of vanA, vanB and vanC genes were detected by PCR. Multi locus sequence typing (MLST) was performed on the vancomycin-resistant E. faecium isolates.

Results: The collection consisted of 45 E. faecium and 16 E. faecalis isolates which originated from 12 different hospitals. Thirty three of 45 E. faecium isolates were vanA positive and the remaining 12 isolates were vanB positive. All but one of the E. faecalis isolates contained the vanA gene (n=15) and the remaining isolates contained the vanB gene. MLST of the 45 E. faecium isolates revealed 10 different sequence types (ST). The STs were ST18 (n=21), ST203 (n=8), ST78 (n=3), ST192 (n=3), ST412 (n=3), ST16 (n=2), ST17 (n=2), ST65 (n=1), ST80 (n=1), and ST306 (n=1). Forty four (98%) of the 45 tested isolates belonged to CC17. The 45 E. faecium isolates originated from nine different hospitals. Each hospital was mainly dominated by one specific ST; however, ST18 was present in three of the nine hospitals.

Conclusion: The vanA gene was the most common in E. faecium isolates whereas vanB was predominant in E. faecalis isolates. Most of the vancomycin-resistant E. faecium isolates belonged to the hospital-acquired clonal complex 17.

Vancomycin-resistant enterococci in haematopoietic stem cell transplant recipients

P.M. Rath*, R. Trenschel (Essen, DE)

Objective: Vancomycin-resistant enterococci (VRE) are emerging pathogens. There are no data on the prevalence in pre-treated patients with haematological malignancies admitted to a BMT unit. Therefore, the prevalence of VRE was determined by using perianal swabs and the clonality of isolates was studied. In addition, follow up isolates were compared with those of the perianal swabs.

Materials: 137 patients were screened for VRE at admission by using perianal swabs cultured on chromogenic agar (Oxoid). Species identification and detection of van A, van B and esp was performed by PCR. Perianal isolates and isolates detected during the hospital stay in routine cultures were genetically compared by PFGE (SmA I).

Results: VRE were detected in the perianal swabs of 18/137 patients (13%). All isolates were identified as E. faecium with van A. Esp was detected in 10 isolates. In seven patients VRE were also detected in other materials during the hospital stay (throat 1 pt., urine 4 pts., central venous catheter (cvc) 2 pts., blood culture 1 pt.). PFGE analysis revealed 25 different genotypes in total. In two patients identical isolates were cultured from perianal swabs. Comparing the isolates of the perianal swabs and those of other samples showed that two patients had an identical isolate in urine (1 pt.), and on a cvc (1 pt.), respectively. In one patient with a positive blood culture the isolate was different to that of the perianal swab. Regarding overall survival and treatment related mortality, clinical outcome of VRE carriers was similar non-carriers.

Conclusion: In this study 13% of BMT patients were colonised with VRE but invasive infections were rare (1 patient). The epidemiology was
polyclonal. In five of seven patients with the additional detection of VRE in other materials than perianal swabs, different genotypes were found.

**P846** In vitro antimicrobial sensitivity trends of enterococci isolated at an Italian teaching hospital. A 2004–2007 prospective report including over 2,700 examined microbial strains

R. Manfredi*, A. Nanetti (Bologna, IT)

**Introduction:** The increasing temporal trend of antimicrobial resistance among Gram-positive cocci (including Enterococci) is of concern, especially among inpatients.

**Materials and Methods:** The temporal trend of the in vitro antibiotic susceptibility rates was examined for all Enterococcus faecalis and Enterococcus faecium strains, isolated at our General Teaching Hospital during the years 2004–2007. The same pathogen isolated more than once from one patient within one month, has been considered once.

**Results:** Among Enterococcus faecalis isolates (2,736 strains tested on the whole), the greater activity rate was achieved by linezolid (100% of tested strains), followed by teicoplanin (97.9–100%), nitrofurantoin (96.4–98.3%), vancomycin (81.0–100%), ampicillin (90.2–91.9%), penicillin (88.8–91.5%), while irregular variations of sensitivity occurred over time for gentamicin (>60% of tested strains), streptomycin (>70% of strains), and tetracyclines (<20% of strains). When considering Enterococcus faecium strains (626 overall isolates), only linezolid maintained a 100% in vitro activity, followed by teicoplanin (87.7–100% of tested strains), vancomycin (78.4–86.2% of strains), tetracyclines (56.8–81%), and gentamicin (59.1–71.0%), while unpredictable efficacy was shown by streptomycin (27.6–69.8% of tested strains). Sixty-six strains of vancomycin-resistant Enterococcal strains were detected, with a clearly increased trend from 2004 (7 cases) to 2007 (21 cases) (p < 0.001). An increased in vitro resistance rate was also detected for tetracyclines, during the four-year study period (p < 0.01).

**Conclusions:** A prospective surveillance monitoring of the in vitro antimicrobial sensitivity figures of Enterococci as relevant hospital pathogens, plays an useful role to target antimicrobial treatment and prophylaxis strategies, on local and regional basis. The emerging of resistance to the reference compounds, and that of vancomycin-resistant organisms in particular, may be also well assessed on these temporal basis, in order to address the clinical choice according to the local epidemiology and antimicrobial testing features.

**P847** Prevalence of vancomycin-resistant enterococci in paediatric patients who referred to a paediatric hospital, Tehran, Iran


**Introduction:** Vancomycin is one of the most effective antibiotics against the Gram positive cocci. Prevalence of resistance between enterococci and its simple spread way to other Gram positive cocci such as staphylococci and streptococci have led to serious problems for in-bed patients. It could be suggested that frequency of resistant enterococci indicate a reliable pattern of antibiotic susceptibility. Result in saving time and cost.

**Objectives:**
1. Prevalence of vancomycin resistant enterococci in Moed children hospital and Ali Asghar hospital
2. Antibiotic resistance patterns in enterococci
3. How is the vancomycin resistance between enterococci isolated from patient's stool samples
4. Pattern of antibiotic resistance of enterococci against other antibiotics
5. Determination of vancomycin resistance and its relation to effective antibiotics for anaerobic bacteria
6. Prevalence of vancomycin resistance in enterococci isolated from stool samples from distinct wards in hospitals.

**Method:** Stool Sampling was performed on in-bed patients in distinct wards in hospital including GI and infectious of Moedegh hospital and chemotherapy in Aliasghar hospital. Stool culture was done once introduce to laboratory 48 hours after patient's admittance. Specimens were cultured on media such as Enterococcagel agar and Bile Esculin agar. And then some specific tests were performed for their confirmation such as PYR test, growth in 15°C and 45°C in Mueller Hinton agar. Growth in 6.5% NaCl. E-test was performed on VRE strains. Selected antibiotics for E-test were included cephapetin, rifampicin, gentamycin, ciprofloxacin, ceftriaxone, oxacillin, cefotaxim, amikacin, clindamycin, chloramphenicol, imipenem. Susceptibility level was reported on the basis of NCCLS charts.

**Results:** In this research, we examine stool samples of 64 patients who had one or two samples. 13 percent of enterococci were resistant. Antibiotic pattern was cefotetan 60%, rifampicin 36%, gentamycin 36%, ciprofloxacin 32%, tetracycline 53%, ceftriaxone 86%, oxacillin 65%, cefotaxime 76%, amikacin 48%, clindamycin 23%, chloramphenicol 24%, imipenem 25%.

**Conclusion:** Antibiotic resistance in enterococci is increasing even vancomycin which is the first choice for enterococcal infections treatment, it could be suggested before effective treatment, disc diffusion test and E-test should be done.

**P848** Nosocomial outbreak of an extended-spectrum β-lactamase producing Klebsiella pneumoniae strain on a medical intensive care unit

R. Laffer*, M. Michoi, M. Buehlmann, C. Mohr, T. Bregenzer, H. Fankhauser (Aarau, CH)

**Objectives:** The incidence of extended spectrum β-lactamase producing (ESBL) bacteria is increasing. Isolation precautions are recommended to prevent transmission. Delayed diagnosis of ESBL colonisation may increase the risk for nosocomial outbreaks. We report an outbreak of ESBL producing Klebsiella pneumoniae (ESBL-KP) on a 10-bed medical intensive care unit (ICU) and surveillance and infection control measures taken to interrupt transmission.

**Methods:** All infected or colonised patients were isolated and cohorted if possible. Hand hygiene was reinforced and surveillance cultures were performed (rectal swabs every 2nd day in all patients who stayed at the ICU for ≥24 hours, environmental sampling from computer keyboards, telephone receiver, water tap, rinsing tank and the lavatory). The number of beds was reduced to a fixed key according to the available staff.

**Results:** The first patients were diagnosed on July 8th. Three additional patients were diagnosed on July 16th. The time between screening and isolation was 3 days according to the time needed for microbiological identification. Hand hygiene promotion was reinforced on July 11th. Surveillance cultures were initiated and environmental screening was performed when 4 additional patients were diagnosed with ESBL-KP on July 23rd. ESBL-KP was also cultured from two rinsing tanks. Water taps, rinsing tanks and lavatories were rinsed with sodium hypochlorite. On August 23rd, 2 newly colonised patients were identified. Intensified investigations could not detect further potential sources of ESBL-KP. There were no further ESBL-KP isolated after August 23rd. In total, 12 patients were affected. 7/12 patients died.

**Conclusion:** The source of our ESBL outbreak could not be identified. Standard hygiene precautions and a high level of awareness for hygiene issues reduced the risk of, and subsequently interrupted transmission. Such measures are particularly important since the population at risk has a poor prognosis, supposedly because of comorbidity but probably due to infectious complications too. The role of environmental or staff screening should be investigated.
**P849** Food-borne nosocomial outbreak due to ESBL-producing Klebsiella pneumoniae (SHV-38). Epidemiology and successful control  
E. Calbo*, M. Riera, N. Freixas, C. Nicolas, O. Montsitol, M. Xercavins, J.M. Naca, J. Vila, J. Garau (Terrassa, Barcelona, ES)

**Objectives:** Nosocomial outbreaks of food-borne ESBL producing K. pneumoniae (ESBL-KP) have not been reported. We describe the epidemiology and control of an outbreak.

**Methods:** After the identification of 2 infected patients with ESBL-KP in a medical ward of a 500-bed acute care teaching hospital, an epidemiological investigation was performed. That included: a systematic faecal sampling in patients of medical, surgical and critical care units, hospital kitchen environmental surfaces and food stuff cultures, and faecal samples of health care workers and food handlers. Characterisation of the ESBL was performed by PCR and sequencing whereas the epidemiological relationship of the isolates was carried out by PFGE.

**Results:** From June-October 2008, 153 colonised/infected patients were identified; 32 patients were infected (21%) (UTI (25), SSI (5), pneumonia (1)) and primary bacteraemia (1)). Given the fact of the high prevalence of faecal colonisation (up to 37% in some wards), the rapidity of its spread, the early colonisation soon after admission, and the lack of carriers among health care workers investigations were directed to the hospital kitchen and food chain. In the kitchen, up to 35% of studied surfaces or foods were contaminated. Six out 44 (14%) of asymptomatic food handlers were found to be faecal carriers. One of the kitchen washing rooms was found to be persistently colonised and justified the persistence of the outbreak over time. Phenotypic and genotypic analysis of all isolates showed that all strains were identical and that the outbreak represented the spread of a single clone of SHV-38 producing KP.

**Conclusions:** Contact isolation measures were immediately applied on colonised/infected patients. A protocol for routine detection and isolation of patients with faecal colonisation was implemented. A proactive campaign to reinforce hand hygiene practices and structural and functional cleaning measures in the kitchen area were carried out. Once the source had been identified and we learned that the food was the vehicle of the massive colonisation of inpatients, isolation practices for colonised patients were abandoned. No restrictions in the use of antimicrobials were needed to control the outbreak. No new cases of nosocomial colonisation/infection were identified after October 2008.

**P850** Environmental contamination in the rooms of ESBL-colonised patients  
H. von Baum*, S. Jung, A. Moericke (Ulm, DE)

**Objectives:** The presence of ESBL positive enterobacteria in the rooms of ESBL colonised patients and its relationship to the contamination of healthcare workers’ (HCW) gloves and clothing was examined.

**Methods:** 12 standardised environmental samples from patients’ rooms as well as samples from the gloved hands and clothing of HCW were examined. Samples were incubated for 48h at 57°C. Species identification of Gram negative bacteria was performed using the API 20E (bioMerieux). Confirmation of ESBL positive isolates was done by standardised susceptibility testing. All ESBL positive isolates were either E. coli or K. pneumoniae. All environmental ESBL isolates as well as the corresponding patients’ isolates were genotyped using pulsed-field-gel-electrophoresis (PFGE) after digestion with the restriction enzyme XbaI.

**Results:** ESBL positive enterobacteria were present in the rooms of only 8 of 36 patients colonised with ESBL positive enterobacteria (22%). Contamination of HCW’s gloved hands and/or clothing was identified in 4 of 65 nurses (6%); 1 of 8 physicians (13%) and 1 of 76 medical students (1.3%). Contamination of physiotherapists, members of the housekeeping staff or visitors could not be found. In only 1 patient more than one ESBL strain was present.

**Conclusion:** Healthcare workers must be aware that contamination of their gloved hands and clothing whilst caring for an ESBL colonised patient is possible. In contrast to the findings for MRSA colonised patients (i) the environment of ESBL-colonised patients is less frequently contaminated, (ii) the contamination occurs usually only in close proximity of the patient and (iii) in the majority of cases the strains from the environment are identical with the clinical isolates of the patients’.

**P851** Transmission rate of Enterobacteriaceae producing extended-spectrum β-lactamase to hospital contacts and household members in a Swiss university hospital  
B.Y. Betocch*, S. Droz, K. Mühlemann (Bern, CH)

**Objective:** Gram-negative bacteria with extended-spectrum β-lactamase (ESBL) production are spreading rapidly world-wide. We studied the transmission rate of ESBL germs from index patients with ESBL carriage to hospital room mates and household members.

**Methods:** Patients with ESBL carriage newly detected during diagnostic work-up for infection were recruited prospectively at the University Hospital Bern during the time period May 7 to December 13 2008. Hospital contacts were defined as room-mates of the index for 48 hours. Screening was performed weekly for the duration of contact and continued for another 2 weeks after separation of the index patient. Screenings were stopped after 2 negative results. Screening included faecal samples for all contacts and respiratory tract sample in patients with intubation or tracheostoma, dermal swab in case of skin lesions and any bodily fluid drained by a catheter. Faecal samples were collected from household contacts in a 3-monthly interval until both index and contacts screened negative. Stool samples were analyzed with 3 different ESBL selective culture media: ChromID ESBL agar (BioMérieux®) and ESBL agar (AES®), a bi-plate with 2 selective media (MacConkey agar plus Ceftazidim and Drigalski agar plus Cefotaxim).

**Results:** A total of 37 index patients, 24 (65%) inpatients and 13 (35%) outpatients were analyzed. The ESBL-species detected was E. coli, K. oxytoca and K. pneumoniae in 58%, 29% and 13% for inpatients and 77%, 15% and 8% for outpatients. Faecal colonisation was detected in 75% of inpatients and in 54% of outpatients. The screening was not interpretable due to antibiotic treatment in 4 index cases and faecal screening was missing in 4 cases. Household screening was performed for 27 members of 16 households. ESBL carriage was detected in 5 households (with 7 members). 3 of these 5 households were of South-Asian ethnicity. Faecal ESBL carriage index patients was 80% in the 5 households with ESBL-transmission and 55% in the 11 households without transmission.

**Conclusions:** The rate of faecal colonisation with ESBL tends to be higher for hospitalised patients than for outpatients (75% vs. 54%). Inhospital patient-to-patient transmission rates may be lower than transmission rates within households and may correlate with faecal carriage in the index patients.

**P852** Skin colonisation with extended-spectrum β-lactamase producing Enterobacteriaceae  
M. Buchelmann*, R. Laffer, H. Funkhauser, T. Bregenzer (Aarau, CH)

**Objectives:** Extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL) are emerging worldwide. ESBL carriers are an important source of ESBL spread and no effective decolonisation is available. Screening for ESBL has been performed by rectal and urine sampling. Skin colonisation of ESBL has not been studied. The aim of this study was to determine the rate of inguinal colonisation in ESBL carriers.

**Methods:** From November 2006 to November 2008 all newly detected ESBL carriers at the Cantonal Hospital of Aarau, Switzerland, were screened by rectal, bilateral inguinal swabs and uricul. ESBL was mainly diagnosed in clinical samples, six patients were screened for ESBL.
Nasocomial outbreaks and potential sources of infection

during a limited outbreak of *K. pneumoniae* ESBL on the intensive care unit involving 14 individuals. Laboratory diagnosis of ESBL was made according to the guidelines of the Clinical Laboratory Standard Institute (CLSI): cefotaxime, ceftazidime, ceftriaxone, and cefazidime were used for screening. Since July 2008, a chromogenic medium (chromID ESBL, Biomérieux, France) has been used for rapid identification of ESBL. If screening was positive, confirmation was performed by E-test strips.

**Results:** Within the study period 65 ESBL carriers with a mean age of 58 years were identified. Of those 46% (30/65) were males and 52% (22/65) were hospitalised at the time of ESBL diagnosis. *E. coli* and *K. pneumoniae* were the most common ESBL types (51% and 47%, respectively), 4 patients were colonised with two different ESBL strains. Inguinal colonisation was found in 64%, rectal colonisation in 70% and urine colonisation in 75% of patients. Inguinal colonisation was more common in hospitalised than in outpatients (75% vs. 25%, OR 8.4, 1.4–47, p = 0.013). In one patient inguinal swabs were the only samples positive for ESBL.

**Conclusion:** Inguinal colonisation is common in ESBL carriers. Skin colonisation may be important for nosocomial ESBL transmission. Future decolonisation attempts should include treatment of skin colonisation with antiseptic soaps.

**P855** The extended antibiogram, a useful tool for typing of ESBL producing *E. coli*

A. Porczak*, M. Sandquist, G. Kahlmeter (Växjö, SE)

**Objective:** Outbreaks of extended-spectrum β-lactamase (ESBL) producing *E. coli* are becoming an everyday problem for local microbiology laboratories and infection control personnel. A simple and rapid method to suggest or exclude epidemiological relationship is therefore needed. Two phenotypic methods can be used without specialised competence in molecular biology. The PhenePlate™-system utilises the dynamics of eleven biochemical reactions over 48 h. The extended antibiogram, in this study comprising 32 antibiotic discs, compares inhibition zone diameters of isolates from two different outbreaks were analysed using PhenePlate™-system and the extended antibiogram. *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 were used as control strains in all systems. Susceptibility testing was performed using 32 antibiotic discs on IsoSensitest agar (Oxoid, Basingstoke, UK). All zone diameters (mm) were registered and antibiograms based on zone diameter values constructed. The PhenePlate™-system was used according to the manufacturers description. Cluster analyses were performed using the PhpWin 4.2 software utilising pairwise comparison statistics.

**Results:** Both methods identified the outbreak related isolates. Both methods identified three distinct clusters. One of the reported outbreaks turned out to consist of two separate outbreaks. The three clusters were verified using PFGE. Neither of the two methods showed unexpected clusters in isolates that were expected to be non-related. However, both methods did occasionally include single isolates unrelated to the outbreak, in the three outbreak clusters.

**Conclusion:** The extended antibiogram and the PhenePlate™-system performed equally well in identifying and excluding outbreaks of ESBL-producing *E. coli*. The extended antibiogram was fast, simple and easy to standardise and we suggest that it can give early and rapid support in outbreak investigation.

**P854** The prevalences and risk factors of nosocomial infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Thailand

N. Hiransathikul†, P. Saonuam, C. Suankrutray, K. Malatham, S. Danchaisirit (Bangkok, TH)

**Background:** Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (E. coli) and *Klebsiella pneumoniae* (K. pneumoniae) have been increasingly reported as causative pathogens of nosocomial infection (NI) in Thailand.

**Objectives:** To determine the prevalences and risk factors of NI caused by ESBL-producing *E. coli* and *K. pneumoniae* in Thailand.

**Methods:** The cohort study and nested case-control study were conducted at 6 regional hospitals and 6 provincial hospitals in Thailand during 1 July 2007 to 31 December 2007. We collected the data of all NIs and those caused by ESBL-producing *E. coli* and *K. pneumoniae*. Nested case-control study were done by using 131 matched pairs of NIs caused by ESBL-producing and non-ESBL-producing *E. coli* and 110 matched pairs of NIs caused by ESBL-producing and non-ESBL-producing *K. pneumoniae*. The hospital where data were collected and the organism were selected as matching criteria.

**Results:** The prevalences of NIs caused by ESBL-producing *E. coli* and *K. pneumoniae* were 59.8% and 59.3%. For subjects with NIs caused by ESBL-producing *E. coli*, the mean age was 61.3 years old with 54% female. The three most common sites of infection were respiratory tract (45.0%), surgical site (22.9%), and respiratory tract (17.6%). For subjects with NIs caused by ESBL-producing *K. pneumoniae*, the mean age was 58.0 years old with 36% female. The three most common sites of infection were respiratory tract (66.4%), urinary tract (16.4%), and surgical site (10.0%). By multivariate analysis, the previous use of third-generation cephalosporin was a significant risk factors associated with NIs caused by ESBL-producing *E. coli* (OR = 5.55, 95% CI=2.73–11.29, p-value <0.001) while the previous use of third-generation cephalosporin and aminoglycoside were significant risk factors associated with NIs caused by ESBL-producing *K. pneumoniae* (OR = 3.95, 95% CI=1.94–8.04, p-value <0.001, and OR = 6.85, 95% CI=1.36–25.21, p-value=0.004)

**Conclusion:** Infections caused by ESBL-producing *E. coli* and *K. pneumoniae* were common in Thailand. The previous use of third-generation cephalosporin was a predictor of NIs caused by each organism, while previous use of aminoglycoside was also associated with NIs caused by ESBL-producing *K. pneumoniae*. Appropriate use of third-generation cephalosporin and aminoglycoside should be implemented to reduce the burden of NIs caused by these pathogens.

**P853** Report of an outbreak of carbon dioxide dependent methicillin-resistant *Staphylococcus aureus* on a hospital ward


**Objective:** To investigate and control an outbreak of carbon dioxide (CO2) dependent methicillin resistant *Staphylococcus aureus* (MRSA) in a regional liver unit (RLU).

**Methods:** On 15th March 2008 a liver transplant recipient was screened for MRSA on admission to the critical care unit from the RLU. Small poorly-growing green colonies were isolated from a nasal swab on chromogenic MRSA identification media (chromID MRSA, bioMérieux) after 24 hours aerobic incubation. They were slide-coagulase positive (Sldex Staph-Plus reagent, bioMérieux). The strain repeatedly failed to grow after 24 and 48 hours aerobic incubation on IsoSensitest agar (IST, Oxoid) or 5% horse blood agar (BA, TSC) for susceptibility testing. When subcultured onto BA and incubated overnight at 37°C in 5% CO2, however, a heavy growth of an isolate with colonial morphology typical of *S.aureus* was seen. Susceptibility testing performed on IST according to British Society for Antimicrobial Chemotherapy guidelines but in CO2 enriched atmospheric conditions (5% CO2) confirmed that this was methicillin resistant *S.aureus* (MRSA), also resistant to erythromycin, clindamycin, moxifloxacin and trimethoprim.

A possible outbreak was suspected as the medical microbiology/infection control teams were aware of another liver transplant recipient on the RLU who was found, in February 2008, to be colonised/infected with a strain of MRSA that grew much better in 5% CO2 than aerobically. All patients and staff on the RLU were screened for carriage of CO2 dependent MRSA using MRSA ID media in 5% CO2. A deep clean of the ward was carried out and infection prevention and control practices were reinforced.
Results: Four further cases (3 patients, 1 staff member) were found to be colonised at one or more sites by a CO2 dependent strain of MRSA. Ongoing targeted screening revealed a seventh case five weeks after the initial outbreak. This patient was admitted to the RLU during March 2008 but had been discharged two days prior to recognition of the outbreak and screening swabs were found to be positive when he was readmitted to the RLU in May. Molecular analysis confirmed that all strains were identical: EMRSA-15 (ST22-SCmecIV).

Conclusions: To our knowledge we report the first outbreak of CO2 dependent MRSA. Similar outbreaks may be missed if screening swabs are processed by conventional methods. Establishing the local prevalence of CO2 dependent MRSA is necessary to determine whether targeted screening is required.

An outbreak of multidrug-resistant *Pseudomonas aeruginosa* sepsis after endoscopic retrograde cholangiopancreatography

J. Kocaie*, N. E. L. Meeussen, F. T. M. Peters, M. H. Been, R. P. Borgers, J. E. Degener (Groningen, NL)

Objective: Endoscopes, including duodenoscopes, are the medical devices frequently associated with outbreaks of nosocomial infections. We investigated an outbreak of multidrug-resistant *Pseudomonas aeruginosa* sepsis affecting 3 patients after endoscopic retrograde cholangiopancreatography (ERCP) during a 4 months period, from July to October 2008.

Methods: Outbreak investigation included microbiological testing of the implicated endoscope and environmental sampling from the washer disinfectors, the connecting tubes and the environmental surfaces in the endoscopy centre. Specimens for culture were obtained from the biopsy/suction and the water/air channels of the implicated endoscope with a retrograde technique. The available *P. aeruginosa* epidemic strains underwent molecular typing by repetitive-DNA-sequence-based polymerase chain reaction (rep-PCR). Results of recent surveillance cultures from endoscopes and medical records of all patients who underwent ERCP with the implicated endoscope were reviewed.

Results: During a 4 months period, from July to October 2008, 3 in total patients developed sepsis with multidrug-resistant *P. aeruginosa* after undergoing an ERCP procedure. Our registration system enabled us to retrieve one of three endoscopes daily in use as the possible source of infection. This ERCP scope demonstrated the contamination with *P. aeruginosa* in 2 surveillance samples in July and September and in 3 consecutive cultures in October 2008. All of the environmental samples and recent surveillance cultures from endoscopes were negative for *P. aeruginosa*. Other 33 patients treated with this endoscope had neither symptoms of infection nor positive blood cultures. Rep-PCR of the *P. aeruginosa* isolates demonstrated matching patterns (95% similarity) and confirmed that this microorganism was transmitted from patient to patient by one endoscope. The implicated ERCP scope was removed and confirmed that this microorganism was transmitted from patient to patient by one endoscope. The implicated ERCP scope was removed and confirmed that this microorganism was transmitted from patient to patient by one endoscope.

Conclusion: This *P. aeruginosa* outbreak was caused by patient-to-patient transmission and infection from a common source, i.e. the flexible ERCP endoscope. Our microbiological surveillance protocol with routine culturing of endoscopes was helpful in detection of the source of contamination. However, the current surveillance system could not prevent the serious infections in three patients and probable numerous cross-contaminations in other persons, who underwent ERCP with this particular endoscope.

Investigation of nosocomial outbreak of multiresistant *Acinetobacter* spp. at a Korean hospital


Objectives: *Acinetobacter* spp. are important nosocomial pathogens with increasing resistance to multiple antimicrobial agents in Korea. Acinetobacter spp. had been the 6th or 7th most frequently isolated species, and imipenem resistance rate was 16% at a university hospital in 2007. In September 2008, laboratory data at the hospital showed sudden increase of *Acinetobacter* spp. isolation. Aim of this study was to determine the source of the spread and to devise measures to control the outbreak.

Methods: Samples taken from instruments and fomites in ICUs as well as from ICU nurses were cultured to find source of *Acinetobacter* infection. Antimicrobial susceptibility was determined by the CLSI disk method. PFGE patterns of Smal-restricted genomic DNA of the isolates from environment and patients were compared to determine the relatedness of the isolates.

Results: Analysis of routine test data showed the number of *Acinetobacter* isolates significantly increased from 44 in January to 160 in September, 2008. Most of the isolates were from ICU patients. Among the 160 isolates in September, 70 (43.8%) were from sputum and other respiratory-related specimens. Resistance rates to relatively more active antimicrobial agents, imipenem, amikacin, and levofloxacin were 40.0%, 46.8%, and 52.5%, respectively. To investigate the source of spread, samples were taken from the environment of ICUs in October. Of the 105 samples, 31 (29.5%) yielded *Acinetobacter* spp. Among these, 28 (90.3%) were from respiratory-related instruments.

Conclusions: Source of outbreak of *Acinetobacter* spp. was mostly respiratory-related instruments in ICUs. Multiresistance may also aided the spread. Control of *Acinetobacter* was only partially successful when judged immediately after the enforced intervention, suggesting requirement of continued efforts.

Outbreak of *Burkholderia cepacia* in two German university hospitals caused by contaminated prefabricated washcloths

M. Martin*, B. Christiansen, I. Chaberny, F. Mattner (Lubeck, Kiel, Hanover, DE)

Introduction: We investigated an outbreak with *B. cepacia* in seven different intensive care units (ICU) of two German university hospitals started in the mid of July 2008. The two university hospitals in the North of Germany merged in 2003. Each hospital has its own infection control team (ICT).

Methods: Cases were defined as microbiologic detection of *B. cepacia* in any material sent to the laboratory. Patients charts were reviewed. Mineral water, different alcohol-free mouthwashes, surfaces of patients environments and moist prefabricated washcloths were investigated for *B. cepacia*. To prove clonal identity of *B. cepacia* strains pulsed-field gel electrophoresis (PFGE, Spel digest) was performed.

Results: In total 41 cases were diagnosed with *B. cepacia* in 7 ICUs and 2 wards of two hospitals. 30 were positive in respiratory specimens, 6 in wound (2 tracheostoma) swabs, one each in vaginal or lip swab, one each in urine, pleural effusion or blood culture. 32 patients were intubated and at least 8 patients had a ventilator-associated pneumonia due to *B. cepacia*. One patient suffered an infection of a pacers cable insertion site. 8 patients died, one probably related to *B. cepacia* infection. Environmental research was started especially looking for liquid and moist equipments. *B. cepacia* was found in opened and closed packages of moist prefabricated washcloths. After recognition of the source German health care authorities were informed and a Europe-wide alarm (RAPEX) was given through the systems to prevent infections in other hospitals. PFGE proved the identical clone in clinical specimens and washcloths of both hospitals. After elimination of the washcloths in both university hospitals no more cases occurred.
Nosocomial outbreaks and potential sources of infection

Conclusion: Contaminated moist prefabricated washcloths were identified as cause of a *B. cepacia* outbreak on seven ICUs of two German hospitals. For critical ill patients it should be carefully reconsidered what kind of care products are used. In case of infections due to contaminated drugs, medical products or cosmetics the immediate information of health care authorities is required to prevent further cases in other hospitals. To spread the information different international alarm systems are established. In Germany, in case of cosmetics the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit is the authority which has to be informed and initiates a Europe-wide warning over the alarm system called RAPEX.

P859 Tap water as a potential source of nosocomial *Pseudomonas aeruginosa* infections in an intensive care unit

Z. Barna*, K. Antmann, J. Párizti, M. Németh, R. Bánski, M. Vargha (Budapest, HU)

*Pseudomonas aeruginosa* is a frequent cause of nosocomial infections in intensive care units. The water distribution system, tap and shower points of use may often serve as reservoirs for pseudomonads. If tap water is identified as the source of infections, appropriate preventive measures can be employed to reduce infection rates.

Aim of the present study was to assess the effect of point-of-use filters on 1. *Pseudomonas aeruginosa* counts in tap water, 2. incidence of *Pseudomonas* spp. infections in an ICU, 3. clinical and environmental *Pseudomonas* spp. strains, in order to provide evidence for water-related infection.

The study was carried out in a 12 bed intensive care unit of a Hungarian hospital. Point-of-use filters (Pall Medical) were applied to all water outlets for 2 × 2 weeks. Tap water was sampled weekly before, during and after the use of the filters. *Pseudomonas aeruginosa* was enumerated according to ISO 12780 standard. Environmental *Ps. aeruginosa* strains were isolated from tap water and compared to clinical isolates by serology, phage and antibiotic resistance profile, pyocin production pattern and total genome restriction pulse field gel electrophoresis (PFGE). Three of five tap outlets were found to be initially colonised by *Ps. aeruginosa* (1–300 CFU/L). Application of the point-of-use filters eliminated *Ps. aeruginosa* as well as other waterborne bacteria from the tap water during the two weeks of usage (as specified by the manufacturer). After the removal of the filters, *Pseudomonas* spp. counts within the water samples returned to the levels initially detected. There were no new clinical cases of *Pseudomonas* infections identified during the use of the filters, whereas an average of 6 cases/month was recorded during the preceding 2 years.

Clinical (13) and environmental (101) strains were collected and identified in the study period. Environmental isolates clustered into two groups by all of the employed typing methods; serotypes O1 and O17 were discerned. All strains (including clinical isolated) had two groups by all of the employed typing methods; serotypes O1 and O17 were discerned. All strains (including clinical isolated) had identical antibiotic resistance profile, all were sensitive to amikacin, 46.7% to ciprofloxacin, 72.2% to gentamicin and 94.4% to aztreonam. Only 4 isolates were sensitive to all 9 drugs, while 59 (65.6%) were resistant to ß-3. Nine isolates were resistant to ß-3 drugs.

Conclusions: Tap water can act as a reservoir for *S. maltophilia*, and some of these environmental isolates are highly multi-drug resistant. This should be taken into account when developing infection control strategies for preventing sporadic infections or outbreaks amongst high-risk patients.

P860 Prevalence and antibiotic resistance of *Stenotrophomonas maltophilia* in the water supply of a haematology ward

C.L. Wright*, K.G. Kerr, L. Newton, A.M. Snelling (Bradford, Harrogate, UK)

Objectives: *Stenotrophomonas maltophilia* is emerging as a significant opportunistic nosocomial pathogen. Patients undergoing therapy for haematological malignancy are at particular risk. Antimicrobial therapy of infection is often complicated as isolates may manifest multi-drug resistance. Outbreaks of *S. maltophilia* have been linked to water sources in hospitals, but little is known about the prevalence and antimicrobial resistance patterns of the bacterium in potable or bathing water. We investigated the longitudinal prevalence of *S. maltophilia* in the water distribution system of a haematology ward and the antibiotic resistance characteristics of isolates.

Methods: Water samples (100 ml) and swabs were taken from the outlets of a 14 bed (12 patient rooms) haematology unit. Samples were collected bi-weekly from each tap or shower in the patient rooms, the kitchen taps supplying cleaning and drinking water, and 2 handwash stations, totalling 38 outlets. Membrane filters of water were incubated for 48 h on R2A agar. Isolates were subcultured on vancomycin-imipenem-amphotericin B agar for selection of *S. maltophilia*. Identity was confirmed by species specific 23S rRNA-PCR. Susceptibility to a panel of 9 common anti-pseudomonal drugs was investigated using disc diffusion.

Results: Over 19 weeks, 647 water samples and 332 outlet swabs were collected. Of these, 74 water samples (11.4%) and 25 swabs (7.5%) yielded *S. maltophilia*. Outlets in 10/12 patient rooms yielded *S. maltophilia* on at least one occasion, but the kitchen and handwash stations were consistently negative. The most persistent source of *S. maltophilia* was showers in patient rooms. There were only 2 weeks when the pathogen was not isolated. Of the 90 isolates examined, none were resistant to minocycline or levofloxacin (using CLSI breakpoints for *Stenotrophomonas*). Using BMAC breakpoints for *Pseudomonas*, 5.6% were resistant to colistin, 25.6% to cefazidime, 30% to ciprofloxacin, 46.7% to amikacin, 62.9% to piperacillin/tazobactam, 72.2% to gentamicin and 94.4% to aztreonam. Only 4 isolates were sensitive to all 9 drugs, while 59 (65.6%) were resistant to ß-3. Nine isolates were resistant to ß-3 drugs.

Conclusions: Hospital tap water can act as a reservoir for *S. maltophilia*, and some of these environmental isolates are highly multi-drug resistant. This should be taken into account when developing infection control strategies for preventing sporadic infections or outbreaks amongst high-risk patients.

P861 Prevalence of *Legionella* spp. in water systems in German hospitals

E.B. Kruse*, S. Schulz-Stuebner (Freiburg, DE)

Objectives: Although there is mandatory testing of drinking water for *Legionella* in German hospitals, the actual prevalence of *Legionella* contamination in the hospital setting remains unclear. In this study, we investigated the prevalence of *Legionella* in water systems in hospitals in Germany in order to determine the risk of exposure and the efficacy of disinfection measures.

Methods: In a nationwide survey, 77 random hospitals were asked to report the results of their last water sampling and, if *Legionella* was found, the control sampling six months later, and the measures of disinfection and/or patient protection which were taken.

Results: Twenty-eight hospitals with 78 to 477 beds answered the questionnaire. At the first sampling, a mean of 9.4 (1–34) samples were taken per hospital. Two hospitals were free of *Legionella*. Twenty-six hospitals had at least one positive sample. Overall, 94 of 260 samples (36.2%) were tested positive. Rates were significantly higher in smaller hospitals (<200 beds) with 56 (50.5%) positive samples out of 111, than in larger ones with 38 (25.5%) positive samples out of 149 (p = 0.0001). Seven (27%) institutions used water filters or restricted the use of tap water for patients at risk. Twenty hospitals (77%) applied at least one additional disinfection measure (see table 1). At the control sampling, one of 14 samples taken at the two previously negative hospitals was positive (7.1%). At the hospitals who had taken additional disinfection measures, 80 of 204 samples were positive for *Legionella* (39.2%). In hospitals without additional measures, 11 of 47 samples (23.4%) were tested positive. Together, *Legionella* was found in 92 of 265 water samples (34.7%). There was no statistically significant difference between the first and second sampling (p = 0.75).

Conclusion: This survey shows that *Legionella* can be found in nearly all hospital water systems at times. Even radical disinfection measures were not generally effective in reducing the *Legionella* burden. Therefore
restrictive use of tap water for immunocompromised patients and those with swallowing problems remains essential. To achieve a more precise estimate of the epidemiological situation in hospital water systems the results of expensive mandatory water tests should be collected and evaluated in a nationwide database instead of vanishing in the archives of local health authorities.

Table 1. Overview of positive samples and measures taken at different hospitals

<table>
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<th>First sample (pos/total)</th>
<th>Measures taken a b c d e</th>
<th>Second sample (pos/total)</th>
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Measures: (a) Technical check of the water system; (b) periodical heating of complete system; (c) constant increase of temperature above 60°C; (d) single heating cycle of complete system plus flushing of all taps; (e) addition of chlorine or chlorine dioxide. n/a, not available. All p-values calculated with Student’s t-test.

**Results:** The majority of the isolates belonged to *Legionella pneumophila* serogroup 1. Molecular typing indicated the presence of three prevalent types of *L. pneumophila* Wadsworth (Type 1: 28/91. Type 2: 51/91. Type 3: 2/91) with different chlorine susceptibility. Type 1 and 2 were pre-existing chlorination, Type 1 being initially predominating, while Type 2 became widespread over the years. Type 3 was isolated only occasionally. Type 2 was fully susceptible to chlorine, while Type 1 and 3 were chlorine-tolerant. Following exposure to chlorine, ATP levels correlated well with the measured number of viable cells of the three Types, but the luminescence signals were significantly decreased only for Type 2, compared to untreated controls.

The WSP allowed to control the spread of *Legionella spp*. in the hospital water distribution system by the adoption of an integrated disinfection-filtration strategy, presumably due to the application of filters at those points-of-use where Type 1 persisted despite chlorination. The predominance of Type 2 in the last period of environmental monitoring could be explained either by the inability to obtain effective chlorine concentration at every point of use or by the emergence of a resistant Type 2 phenotype.

**Conclusion:** Standard environmental surveillance methods may not be sufficient to choose the most effective and efficient water safety plan, and should at least in some instances be accompanied by in vitro evaluation of the susceptibility of the environmental isolates to the sanitising agent considered.

**Results:***

Use of soda fountains in hospitals: four-year surveillance of daily routine

D. Luft, A. Conrad, B. Balbikir, A. Neuwöhner, R. Scholz, M. Schuhmacher, M. Dettenkofer* (Freiburg, DE)

**Objectives:** Soda fountains (cooled and carbonated water produced directly from the hospital tap water supply) are a convenient way to provide patients, employees and visitors with drinking water. Use of such devices can reduce required storage space, waste and transport-related emissions compared to the classical supply with bottled mineral water. However, microbial contamination of water from soda fountains can occur and may pose a threat to immunocompromised patients. Fifty-one soda fountains are currently in use at the University Medical Center Freiburg and under continuous surveillance regarding microbial water quality.

**Methods:** Water samples were obtained routinely after installation of new soda fountains and twice a year thereafter. Microbiological results were evaluated according to the German drinking water regulation (GDWR). If the acceptable number of colony forming units (CFU) was exceeded (>100 CFU/ml at 22°C; >20 CFU/ml at 36°C), water analysis was repeated with a new sample. If a second sample was still positive, or if pathogenic bacteria or high colony counts were detected in the 1st sample, the fountain was put out of service until a negative sample was obtained after mandated maintenance and disinfection.

**Results:** From 2004 to 2008, 358 water samples from 51 different soda fountains were analysed. The mean number of samples per fountain was 7 (1–15). 309 samples (86%) met the criteria of the GDWR, the remaining 49 (14%) did not. Of the latter, 43 samples (88%) exceeded the acceptable number of CFU, 5 samples (10%) were positive for coliform bacteria (3) or *P. aeruginosa* (2), 1 sample (2%) was both positive for coliforms and had excessive CFU counts. No enterococci or *E. coli* were found. Additionally (not contained in GDWR) we identified *A. baumannii* (2 samples), *S. maltophilia* (2) and yeast (1). The rate of non-conform samples dropped steadily from 25% (16 out of 64) in 2004 to 3% (2 out of 77) in 2008.

**Conclusions:** State-of-the-art soda fountains are a feasible alternative or addition to bottled mineral water. However, standard cleaning, disinfection and maintenance procedures should be implemented to ensure safe operation. Continuous surveillance should be performed to identify deficient fountains. Use of these devices in high-risk areas is not recommended since pathogens found in our samples may endanger immunocompromised patients. Replacing charcoal filters with particle filters may have contributed to the decrease of non-conform samples.
**Evaluation of recent cases of carbapenem-resistant organisms in Hong Kong**

By J. Ling, K.T. Wong, T. Ling

**Objectives:** To document the prevalence of carbapenem-resistant organisms in stool samples in Hong Kong.

**Results:** The incidence of microbial contamination of in-use eye drop products was 17.8%, with the highest rate (24.6%) and the lowest rate (9.0%) noted with day 1 and day 3 samples, respectively.

**Conclusion:** A total of 53 (2%) strains were that imipenem-resistant and belonging to six species were obtained with 37 (70% of the 53) being Stenotrophomonas maltophilia. The others were Klebsiella pneumoniae (1), Acinetobacter baumannii (9), Pseudomonas aeruginosa (3), Cepecea lapagei (2) and Aeromonas hydrophila (1). The MICs of imipenem to these strains were 16–128 μg/ml. The K. pneumoniae strain was isolated from a patient who had an imipenem-sensitive K. pneumoniae strain isolated from the sputum previously. The A. baumannii strains were from long-term hospitalised patients who had been on various β-lactams. The two Pseudomonas aeruginosa strains were from patients who also had the strains in their sputum or bile and had been on antibiotics that included β-lactams and aminoglycosides. The Aeromonas hydrophila strain was from a patient in the surgical ward after undergoing elective surgery. Both the two Cepecea lapagei strains were from patients who had been recently admitted into the hospital.

**Diagnostic methods**

By A. Marigliano, S. Sacini, E. Manso, P. Barbadoro

**Objectives:** To evaluate the usefulness of a sonication protocol to assess the possible cross contamination within the NICU.

**Methods:** All stool samples from patients of seven hospitals in Hong Kong sent for routine bacterial culture during January to August 2008 were tested. Approximately 500 μg of an undiluted stool sample were spread onto a MacConkey agar plate and an imipenem disk (10 μg) was placed onto the primary inoculum. The agar plates were incubated at 35°C for 24 hours. Colonies growing around the imipenem disk were picked and their susceptibility to imipenem was tested by a disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI). Microorganisms that were resistant were identified by the API system. The minimal inhibitory concentration (MIC) of imipenem was determined by a microbroth dilution method (CLSI). Escherichia coli ATCC 25922 was used as a negative control and a clinical strain previously confirmed to be imipenem-resistant was used as a positive control.

**Results:** A total of 3,138 stool samples from 2,894 patients were examined. The age of the patients ranged from 1 day to 102 years and the male to female ratio was 1:1.15. The incidence of bacterial contamination of in-use eye drop products in hospitals, but we did not find a direct relationship between usage duration and contamination rate.

**Conclusions:** The PFGE profiles of the isolates of cases nº2 and nº3 are indistinguishable from each other, so that we can assume that these strains are genetically and epidemiologically related. Further, we can say that these strains are closely related to the supposed index case (case nº1).

**Diagnostic methods**


**Objectives:** To evaluate the usefulness of a sonication protocol to detect the presence of bacteria in retrieved osteosynthesis implants.

**Methods:** A sonication protocol previously developed by us was used in the experiment (Esteban J et al. J Clin Microbiol 2008; 46: 488–492). The protocol includes both low-power sonication during 5 minutes, centrifugation, the use of a broad spectrum of culture media (including specific media for fungi and mycobacteria), and quantitative evaluation of the results. Osteosynthesis implants were processed within 24 hours from surgical removal. Clinical diagnosis of infection was performed according internationally accepted schemes.

**Results:** Between July 2006 and November-2008, 63 samples from 47 patients (1.34 samples/patient) were processed. Samples included nails (23 samples), plates (10 samples), groups of screws (19 samples) and other osteosynthesis material (11 samples). 15 patients (21 samples) had a clinical diagnosis of infection. Among these cases, 17 samples gave positive results (77.3%). Bacteria isolated from these cases included 9 strains of S. aureus, 3 S. epidermidis, 2 Enterococcus sp., 2 S. intermedia, 1 S. maltophilia, 1 P. stuartii, 1 P. prectosi, and 3 cases with mixed anaerobic bacteria (>3 different species/sample). 8 cases had >1 isolates. Among the clinically non-infected patients (42 samples), 11 samples from 10 patients gave positive results (26.2%).
Isolates included 1 *Burkholderia* sp., 2 *M. fortuitum*, 2 *P. aeruginosa* (from the same patient), 1 *R. picketti*, 1 *S. pascinomobilis*, 2 *S. epidermidis*, 2 *S. aureus*, 1 *C. parapsilosis* and 1 *Micrococcus* sp. Two samples had >1 isolates. The average colony count was 69,927.08 CFU/ml for the samples from clinically infected patients (range: 50–100,000 CFU/ml) and 36,473.08 CFU/ml for clinically non-infected patients (range: 50–100,000 CFU/ml), a statistically significant difference (Student’s T, *p* = 0.02).

**Conclusions:** The presented sonication protocol is a valuable tool for the isolation of bacteria from retrieved osteosynthesis implants. Patients without clinical infection can show higher counts of potentially pathogenic bacteria, although the average count is significantly lower than the average count of the patients with clinical infection. The clinical significance of low pathogenic organisms is doubtful, but they may not be considered as colonisation or contamination without further evaluations.

**P868 Using of sonication of bone or removed orthopaedic prostheses for diagnosis of infection after surgery**

G. Ersoz*, M. Uguz, V. Ozultan (Mersin, TR)

**Objectives:** Culturing of samples of bone or removed orthopaedic material is the standard method used for the microbiologic diagnosis of osteomyelitis. Yet, this method is neither sensitive nor specific. Microorganisms are typically present in a biofilm on the surface of the prosthesis or bone. Trampuz et al recommended culture of samples obtained by sonication of prostheses to dislodge from biofilm or bone surface. We hypothesized that using sonication for culturing of samples obtained from bone or removed orthopaedic material would be more sensitive for the microbiologic diagnosis of osteomyelitis.

**Methods:** Patients with osteomyelitis diagnosed clinically and/or radiologically and underwent orthopaedic surgery for revisions or resections were included in the study. We performed a prospective trial to compare culturing of samples that were obtained peroperatively by sonication of bone and/or prostheses with conventional culture of tissue.

**Results:** Fourteen patients (9 male and 5 female) were included. We cultured ten bone samples, one bone graft and three prosthetic materials. With the use of conventional microbiologic procedure, we defined osteomyelitis in four (28.6%) patients. With sonication, the fluid cultures were positive in ten patients (71.4%) (*P* = 0.031). Means of the colony count numbers before and after sonication were 1000.00 (range: 4.10–1664.10) and 32357.29 (range: 0.00–35334.76) (*P* = 0.004), respectively. Counts as colony-forming units (CFUs) and bacterial identifications were shown in the Table. Before sonication, only one colony of methicillin-resistant coagulase-negative *Staphylococcus* was isolated from one patient and evaluated as contamination. But after sonication 50000 CFUs of the same strain were obtained from the same sample. All cases were cured successfully.

<table>
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<th>Case no.</th>
<th>Before sonication</th>
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<tr>
<td>1</td>
<td>5000 CFUs <em>Pseudomonas aeruginosa</em></td>
<td>50000 CFUs <em>Pseudomonas aeruginosa</em></td>
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<tr>
<td>2</td>
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<td>3</td>
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<td>6000 CFUs MSSA</td>
</tr>
<tr>
<td>4</td>
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<td>100,000 CFUs MSSA**</td>
</tr>
<tr>
<td>5</td>
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<td>No growth</td>
</tr>
<tr>
<td>6</td>
<td>1 CFU MRCONS**</td>
<td>50,000 CFUs MRCONS***</td>
</tr>
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<td>50000 CFUs <em>Pseudomonas aeruginosa</em></td>
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</tr>
<tr>
<td>13</td>
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<td>60000 CFUs <em>Pseudomonas aeruginosa</em> and <em>Enterococcus</em> sp.</td>
</tr>
<tr>
<td>14</td>
<td>No growth</td>
<td>2 CFUs MRCONS</td>
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* Methicillin-sensitive *Staphylococcus aureus*.
** Methicillin-resistant *Staphylococcus aureus*.
*** Methicillin-resistant coagulase-negative *Staphylococcus*.

**Conclusions:** For a better evaluation and approach to the diagnosis of osteomyelitis, data should include clinical, microbiologic, and tissue histopathological findings. Using of sonication technique for isolation of agents of osteomyelitis from bone or other specimens that were obtained peroperatively was more effective than conventional microbiological methods.

**P869 Sonication cultures of explanted components under supposed prosthetic or implant infection**

J. Holinka*, M. Pfeiffer, R. Koitz, A. Hirsch, W. Graninger, E. Prestel (Vienna, AT)

**Objectives:** Microbial biofilms growing adherently on prosthetic surfaces may inhibit the detection of the pathogens causing prosthetic joint infections. To evaluate the usefulness of sonication cultures in our patients with prosthetic joint infections we investigated this promising method, and compared the results to those of periprosthetic tissue cultures and histology.

**Methods:** The sonication cultures of the explanted prosthesis were cultured according to the protocol by Trampuz et al. in the New England Journal of Medicine and using the routine method incubating the aspirated pus and periprosthetic material in brain-heart-infusion broth without sonication. To assess the most frequently affected component of the prosthesis all components were “sono-cultured” separately. The diagnosis of infection was based on the presence of bacteria or leukocytes in pus or tissue plus local signs and symptoms and/or systemic markers of inflammation (fever, leukocytosis, increased C-reactive protein).

**Results:** We investigated 60 patients with 41 septic and 19 aseptically explanted components of total knee (n = 24), hip (n = 21) tumour (n = 6) and shoulder (n = 2) endoprosthesis, as well as osteosynthetic material (n = 6) and spinal instrumentation (n = 1). The most frequently affected component of the hip prosthesis was the inlay and the cup with each 89%, of the knee prosthesis was the polyethylene-inlay with 56%, of the tumour prosthesis was the femur component with 43%, of the shoulder prosthesis was the sphere and stem with 100%, of the osteosynthetic material were the plate and screws with each 50% and of the spine instrumentation were the rod and the screws each with 50%. From all detected pathogens in sonication cultures the most frequently were *Staphylococcus aureus* (29%), *Staphylococcus epidermidis* (25%) and *Corynebacteria* (7%). The sensitivity of sonication cultures and periprosthetic tissue cultures was 85% and 72% (p < 0.001) without preoperative antibiotic therapy compared with histological analysis of 100% sensitivity. The specificity was 89% for sonication cultures, 95% for periprosthetic tissue cultures and 100% for histological analysis.

**Conclusion:** Our results of separating the explanted components for sonication culture proved the detection of valid pathogens for every kind of endoprosthesys or implants and supplied further information for the focus of infection.

**P870 Percutaneous bedside bone biopsy coupled with gallium SPECT-CT in suspected diabetic foot osteitis: a pilot study**


**Objectives:** Diagnosing diabetic osteitis might be difficult as it shares with osteoarthropathy the same clinical and radiological inflammatory features. We have evaluated the association of gallium SPECT-CTs and percutaneous bone biopsies to diagnose osteitis in a diabetic foot clinic.

**Methods:** In a pilot prospective monocentric study, all patients suspected of foot osteitis underwent a gallium SPECT-CT scan (Single Photon Emission Computed Tomography–Computer Tomography), to precisely spot the suspected infection, and apercutaneous bone biopsy performed during the consultation.

**Results:** From may 2007 to november 2008, 36 diabetic patients suspected of osteitis (because of chronic foot ulcer) were enrolled. They all had a gallium SPECT-CT scan to detect an inflammatory bone lesion in regard of the skin ulceration evocative of osteitis. Five patients (14%) did not demonstrate any inflammatory bone fixation, so therefore no biopsy was performed. The other 31 patients demonstrated a bone gallium fixation next to the chronic skin ulcer. A bone puncture with a Mallarne needle was performed. Bone aspiration was inoculated in Bactec® haemoculture aerobic and anaerobic bottles.
Three out of 36 punctures were excluded because antibiotics were started before the puncture. Fourteen punctures (42%) were negative with a good 3 months outcome without antibiotics. Nineteen punctures (58%) were positive: one Streptococcus, twelve coagulase negative Staphylococcus and six Staphylococcus aureus (three MRSA). All cultures were monomicrobial and positive within the first 24 hours. All patients with positive bone cultures received antibiotics adapted to the sensitivity for 8 weeks (including ofloxacin, rifampicin, linezolid), and were evaluated at least three months after. They all were improved with no local inflammatory signs, nor tomographic spotting when available.

**Conclusion:** The coupled procedure, gallium SPECT-CT scan-bone aspiration with Mallarné needle is efficient to diagnose bone osteitis in diabetic feet. Gallium SPECT-CT scan provides a functional and exact imaging of bone inflammation when puncture isolates the responsible micro-organism in case of infection. The main interest remains a bedside procedure after imaging. Coagulase negative Staphylococcus seems to be the most frequent germ found in diabetic osteitis.

**P871** Comparison of Copan eSwab with Copan Venturi Transystem for the quantitative survival of Escherichia coli, Streptococcus agalactiae and Candida albicans

S. Nys*, S. Vigen, K. Magehrman, R. Cartuyvels (Hasselt, BE)

**Objectives:** Swab transport systems should preserve the viability and stability of microorganisms in clinical specimens throughout the transport and storage process. In this study, eSwab, a new nyleon-flocked swab in modified liquid Amies transport medium specially designed to optimise specimen collection and to minimise entrapment of the microorganisms, was compared with an Amies agar swab system for the quantitative survival of Escherichia coli, Streptococcus agalactiae and Candida albicans.

**Methods:** The quantitative elution method, as described in the CLSI document M40-A, was used to evaluate the performance of the eSwab (Copan, 480CE) and the Copan Venturi Transystem (CVT, Copan, 108C USE) in the maintenance of E. coli (ATCC 25922), S. agalactiae (ATCC 13813) and C. albicans (ATCC 90028) after 0, 6, 24 and 48 hours preservation at 4°C. Also different concentrations of E. coli and S. agalactiae (9.1, 7.3, 5.5, 3.7 and 1.9) were tested.

**Results:** The average colony forming unit was for both E. coli and S. agalactiae more than one log higher for the eSwab as compared to the CVT after 6 to 48 hours at 4°C. The recuperation when using the eSwab resulted in a similar colony count compared to the control experiment.

For the combinations of E. coli and S. agalactiae, the eSwab performed overall better compared to the CVT. In the 9:1 ratio after 24 hours at 4°C no S. agalactiae could be cultured from the CVT, whereas the colony count for the eSwab remained stable throughout the experiment (0 to 48 hours at 4°C). E. coli colony counts were for the eSwab also ~1 log higher for all time points tested. For the E. coli and S. agalactiae ratio of 1:9 similar results were found as for both strains separately. For C. albicans no significant differences in the performance of both transport systems were detected. The colony counts of both systems were also similar to that of the control experiment.

**Conclusion:** The eSwab transport system showed overall a similar or even better recovery of all microorganisms tested. Furthermore, preservation of the eSwab for 48 hours at 4°C had no influence on the results of the colony counts. Also, E. coli showed hardly any proliferation after 48 hours conservation in the eSwab transport system.

**P872** Copan ESwab, the first liquid-based microbiology device, preserves microbial viability up to 96/120 hours

A. Giambra*, S. Castriciano, R. Paroni (Brescia, IT)

**Objectives:** Although it is recommended that swabs specimens should be processed soon after collection, delays are occurring due to microbiology laboratory consolidation. Specimens transport to centralised laboratories results in long storage times, samples accumulation and increasing workload. Therefore the use of a preservation medium that supports microbial viability for prolonged storage time is necessary. ESwab (ES), a high release flocked swab combined with one ml of liquid Amies medium, is the first liquid based microbiology (LBM) collection and preservation system that provides a homogeneous specimen suspension and allows multiple testing from the same original sample. The objective of the study was to compare the ability of ES to Amies Agar Gel Transystem (TS) to maintain the viability of a panel of microorganisms for an extended period of time.

**Method:** A panel of ATCC microorganism strains, representative of different body sites infections, was tested. P. anaerobius (PA), S. pneumoniae (SP), B. fragilis (BF), H. influenzae (HI) were selected for the respiratory system; VRE for the gastrointestinal system; S. pyogenes (SPY), P. acnes (PAC) for the skin membrane system; C. albicans (CA), for the genital tract system; MRSA for multi-site infections. Each strain was serially titrated and each swab was loaded with 100μl inoculum, to obtain 300–500 CFU from each time zero (T0) plate. After inoculation, the swabs were held at room temperature (RT) for the first 24h, refrigerated for the following 72/96h and plated every 24h.

**Results:** Colonies count was recorded for each strain for all storage times. HI in ES was viable up to 120h but negative in TS. SP BF, SPY, PA and PAC were stable at 120h in ES; one log reduction was found for SP at 48h, for BF, SPY and PA at 96h in TS. CA, MRSA and VRE were stable up to 120h in both devices, but TS had one log reduction. Testing of additional 10 microorganisms is in progress and results will be reported later. In some cases at T0, recovery rate of ES was up to 20% higher than TS.

**Conclusion:** ES was superior to TS in maintaining bacterial viability for longer time and had a higher colonies count than the TS. The ES with its ability to maintain the original microbial load up to 96/120h can be used for the collection of clinical specimens that require longer processing time, multiple testing and results confirmations.

**P873** Gram-stain by a new specimen collection system: ESwab

C. Fontana*, M. Faivar, D. Limongi, J. Pisonkova, C. Favaelli (Rome, IT)

**Objectives:** The first step in routine microbiology laboratory procedures is the collection and safe transportation of swab samples. This can be accomplished using the Copan ESwab Collection and Transport System which incorporates a flocked swab with a tube of modified Liquid Amies transporting medium. Aim of the present study was to compare the Gram stain exam results of smears prepared from clinical specimens collected and transported in the ESwab with those obtained using Amies Agar Gel without charcoal Transystem.

**Methods:** A total of 80 samples (32 vaginal swabs, 27 cervical swabs, 11 urethral swabs and 10 wound swabs) were collected and examined by Gram-stain. Two swabs were collected from each patient, one using the conventional Amies Gel WO/C Transystem (Copan Italia), the other using ESwab (Copan Italia). Once a swab sample is collected, it was placed immediately into the ESwab transport tube and transported directly to the laboratory. For each specimen two set of slides were prepared: the first using 100 microliter of Amies medium, the second using 50 microliters. Both ESwab-slides were Gram stained using an automated Gram stainer. The microscopic examination results of the ESwab-slides were compared with those obtained by the observation of the slides (one for each sample collected) prepared directly at the time of the sample collection (using Transystem).

**Results:** Microscopic examination of 240 slides from 80 different specimens evidenced that the quality of smear preparation of ESwab-slides are superior to that obtained from conventional specimen collection system. Particularly, those prepared using 100 microliters of ESwab medium evidenced more details either concerning the amount of cellular elements (epithelial cell and leucocytes as well as red blood cells and clue cells) or bacteria/fungi elements. Moreover the slides prepared from ESwab exhibits a very good preservation of cellular elements. The micro-organism elements that are more frequently observed in ESwab slide and not in traditional slide are: yeasts, Gram-negative bacilli and Gram-positive diplococci.
Conclusion: The flocked swab of the ESwab kit demonstrated superior absorption and release abilities of sampled material in the medium as evidenced by the significantly higher counts of cellular as well as microbial elements evidenced on the slide preparations. Herein microscopic exam performed using ESwab, especially when preparing the slides with 100 microl, shows excellent results.

**P874** Evaluation of Sysmex UF1000i, a novel high-performance and high-throughput third-generation flow-cytometry screening method for the exclusion of urinary tract infection

R. De Rosa, M. Acelio, S. Grosso, G. Bruschetta, A. Camporese*
(Pordenone, IT)

**Objectives:** Urinary tract infections (UTI) are the infectious diseases with the highest incidence in the hospital and community population. Although the incidence of UTI is high, a large proportion of the samples tested by a routine microbiology laboratory will show no evidence of infection with up to 80% of the specimens with negative results for urine culture. Therefore a rapid and reliable screening method is useful to screen out negative samples, reducing unnecessary testing.

**Methods:** The Sysmex UF-1000i is a fully automated third-generation flow cytometry analyzer now able to better determine bacterial values with the development of a reagent system which exclusively stains bacteria. Our study investigated 1,298 urine samples collected from inpatients and outpatients and compared Sysmex UF1000i with standard urine culture tested on Cled and CNA agar plated by means of 10 microliter loop.

**Results:** The results obtained are very interesting, especially if UF1000i is used as a screening method for negative urine samples, and comparable to data obtained from culture examination. Considering together bacteria/yeasts and/or leukocyte count (>200 bacteria, >30 yeasts and/or >100 leukocytes/μl) in comparison with the standard culture method, diagnostic performances for Sysmex UF-1000i were: sensitivity 98.7%, specificity 76.9%, negative predictive value 99.5%, positive predictive value 59.7.

**Conclusion:** The results of the present study allowed us to improve the efficiency and effectiveness of the whole diagnostic process on urine samples submitted for microbiological investigation. The high negative predictive value (99.5) and the low percentage of false negatives (less than 0.3% of the total samples analyzed), both absolutely fundamental to guarantee the diagnostic efficacy of the screening process, allow us to claim that the Sysmex UF-1000i is able to reach the diagnostic excellence that we set out to obtain. From the management point of view, one of the most interesting features that we have experienced with Sysmex UF1000i is its major contribution to improving the global turn around time, as 57.5% of samples can be reported as negatives within a few minutes of the sample admission, and can be sent to the Laboratory Information System for validation and subsequent immediate reporting in case of negative results. For physicians this should mean prompt reporting of normal samples and improvement in the quality of patient care.

**P875** The Sysmex UF-1000i flow cytometer as a means of reducing urine cultures

J. L. Díaz de Tuesta, D. Suárez*, J. Sánchez, Y. Martín, R. Cisterna
(Bilbao, ES)

**Objectives:** The gold standard method for diagnosis of urinary tract infection is semiquantitative urine culture, although fewer than 30 percent of urine samples sent to the laboratory are proven positive. Thus, a rapid screening method is required to reduce these time-consuming and expensive procedures. The aim of this study was to evaluate the Sysmex UF-1000i flow cytometer (Sysmex Corporation, Kobe, Japan) as a means of reducing the number of urine samples requiring culture.

**Methods:** A total of 786 urine samples from general practice patients and represented all age groups were collected and analyzed for white blood cells (WBC) and bacteria by the UF1000i flow cytometer (Sysmex). Semiquantitative culture was performed on a culture plate of Chromagar Orientation medium (Becton Dickinson) using a standard 1 ul loop and incubated overnight at 37°C in air. Culture results were reported as no growth; urine contaminated if there were 5 or more kinds of colonies without a dominant species; and were considered positive if they contained $\geq 10^3$ to $<10^4$ colony-forming units/mL (cfu/mL) of an urinary pattern of culture, if two or more potentially pathologival bacterial species were isolated when the individual counts were $\geq 10^5$ cfu, or when the count for one organism was $\geq 10^6$ cfu/mL and it was clearly predominant. The microorganisms isolated were identified and antibiotic sensitivities were determined.

**Results:** Table 1 shows the results of the Sysmex UF-1000i flow cytometer test using 20 WBC/μl and 25 bacteria/μl count as the cut-off compared with bacterial culture.

| Culture positive/sysmex positive | 149 |
| Culture negative/sysmex positive | 339 |
| Culture positive/sysmex negative | 10 |
| Culture negative/sysmex negative | 288 |
| Sensitivity | 94 |
| Specificity | 46 |
| Positive predictive value | 30 |
| Negative predictive value | 96 |

**Conclusions:**
1. These cut-off values for bacteria and WBC give an acceptable Negative Predictive value (96%) and allow for the reporting of negative results without culture being performed, reducing the urine cultures by 38%.
2. This reduces the turnaround times for these samples from 24 h to same-day reporting.
3. A result extremely pathological of the cytometer in a sample with a bacterial culture negative suggests a repetition of the analysis or an enlargement of the study towards other feasible pathogens no detectable through standard bacterial culture.

**P876** Evaluation of three diagnosis models for differentiating bacterial from viral meningitis

V. Cocquerelle, C. Fossard, L. Souply, L. Croce, B. Jaubiac, F. Schramm, J.P. Gut, S. Faft-Kremer, P. Riegel*
(Strasbourg, FR)

**Objective:** Evaluation of diagnostic models for differentiating bacterial meningitis (BM) from viral meningitis (VM): three models in a population of children and one model in a population of adult patients.

**Methods:** Retrospective study design. Analysis of a consecutive series of 102 patients (42 adult patients and 60 children under 15 y) with a diagnosis of community-acquired meningitis in the period from 2006 to 2008. The diagnosis was based on direct Gram stain, cerebrospinal fluid (CSF) culture, blood culture, CSF antigen or CSF PCR for BM and on CSF PCR for VM. For adults, we evaluated the Hoen model or pABM (Hoen et al. EJCMID. 1995: 252–254). For children, this pABM model was also proposed (Jaeger et al. EJCMID. 2000. 418–21) and we evaluated it in comparison with the “Bacterial Meningitis Score” (Nigroglie et al. JAMA. 2007. 52–60) and with the De Cauwer score (De Cauwer et al. Eur. J. Emerg. Med. 2007. 343–7).

**Results and Conclusions:** For adults, the pathogens were N meningitidis (n = 11), S. pneumoniae (15), L. monocytogenes (3), H. influenzae (1), enterovirus (5) and other virus (22). For children, the pathogens were N. meningitidis (8), S. pneumoniae (2), enterovirus (29) and other virus (3).

Routine analyses cannot be used alone to distinguish between BM and VM: CRP >4 mg/L (Sensitivity for adults/children: 100/99, Specificity for adults/children:33/22), CSF protein >0.45 g/L (Sc:97/80 Sp:32/75), CSF leucocytes >10/mm³ with >50% of PMN (Sc:83/90, Sp:100/62),
ratio CSF glucose/serum glucose <0.5 (Se/93/60, Sp/79/93). All the cases but one exhibited at least one abnormal result for these analyses. Use of the pABM in adults can distinguish 19 of 25 patients with VM from BM and allowed to predict BM with 100% accuracy (true positives) and 24% false positives (Se: 100%, Sp: 76%). In contrast, the pABM used in the population of children exhibited only 80% specificity (2 false negatives in patients of 2 and 3 y). The sensitivity of the BMS and the De Cauwer score for cases of BM in children was 100% and the specificity was 62.5% and 47%, respectively. It is noteworthy that one case of BM was detected by assigning the BMS on the basis of a positive Gram stain alone. The pABM is quite reliable for differentiating between BM and VM in adults but not in children. The BMS and the De Cauwer score could be an accurate decision-making tool, ensuring 100% accuracy and limiting the treatment with antibiotics in our population of children.

BacT/Alert automated blood culture system for culturing sterile body fluids and deep site abscesses

A. Christidou*, Z. Gkiti, S. Kanaki, S. Maraki (Heraklion, GR)

Objectives: This study compared the BacT/Alert automated blood culture system (Biomerieux) with the conventional culture method for recovery of microorganisms from sterile body fluids and deep site abscesses.

Material and Methods: Sterile body fluids (pleural, peritoneal, synovial, CSF), or deep site abscesses were cultured using both methods (BacT/Alert system and conventional method). For BacT/Alert system, a pair of an aerobic and an anaerobic bottle was used. For conventional method, the specimens were centrifuged at 3000rpm for 15 min and inoculated on blood agar and chocolate agar plates, and in Schaedler broth.

Results: A total of 225 specimens (41 peritoneal, 34 pleural, 25 synovial, 1 cerebrospinal fluids, and 124 deep site abscesses) were cultured and 137 pathogens were recovered from 104 specimens. Among the 104 positive specimens, microbial growth was detected with both methods in 71 (68%) specimens, with only the BacT/Alert system in 29 (28%), and with only conventional method in 4 (4%). Of the 137 isolates, 71 (52%) were recovered by both methods, 50 (36%) by BacT/Alert only, and 12 (16%) by conventional method only. Polymicrobial growth was detected in 21 specimens and 59 strains were isolated from these specimens. Among them, 21 (36%) were detected by both methods, 26 (44%) by BacT/Alert only, and 12 (20%) by conventional method only. Regarding the isolates, 21% (4/15) of S. aureus, 30% (11/37) of coagulase negative staphylococci, 31% (5/16) of enterococci, 68% (13/19) of streptococci, 31% (9/29) of Gram negative bacteria, and 57% (4/7) of anaerobes were detected by BacT/Alert system only, versus 0%, 5% (2/37), 12.5% (2/16), 10.5% (2/19), 21% (6/29), and 29% (2/7) respectively, that were detected by conventional method only.

Conclusion: BacT/Alert blood culture system was more sensitive than conventional method for detecting bacterial growth and recovery of pathogens, especially in staphylococcal, streptococcal species, and anaerobic bacteria. BacT/Alert system alone, or in combination with conventional method can successfully be used in culturing sterile body fluids and deep site abscesses.

Liquid-based microbiology and automation: a new frontier in the management of bacteriology laboratory

V Graziosi*, A. Lecsi, L. Deflorio (Milan, IT)

Objective: Bacteriology specimens have been historically inoculated and streaked manually onto agar media. Some systems to perform these tasks automatically are now available on the market. These automations can process only liquid specimens. Copan Italia (Copan) has developed a range of collection and transport devices that provide the lab (and automation) with a liquid specimen – a new approach called Liquid Based Microbiology (LBM). An automation called “Walk Away Specimen Processor” (WASP, Copan) performs the inoculation and streaking from a variety of bacteriology specimens (swabs, urine, faeces, etc.), fully managing the opening/recapping of containers and plates-labelling. Our laboratory is beta-testing site for the WASP and the LBM range. We evaluated the quality of the culture results, when processed by the WASP and manually; we report our experience in using the machine.

Methods: Six months prior the implementation of WASP we adopted in our routine ESWab and Uriswab (both from Copan), replacing the traditional collection and transport systems for vaginal swabs (Transystem with gel) and urine (vacuum tubes with preservative). ESWab comprises a flocked swab and a tube with liquid Ames medium, while Uriswab is a container incorporating a sponge bonded with preservatives and a tube; both devices allow manual as well as automatic processing from just one specimen collected. WASP was installed on 29/10/2008 and used to process vaginal swabs and urine. For one month clinical specimens were processed manually and automatically, and results compared, for 832 ESWabs and 1,378 Uriswabs. WASP was evaluated in terms of throughput, reliability, reproducibility; consistency with the results from manual processing was analysed.

Results: The quality of the plates processed automatically is equivalent or superior to those obtained manually. For vaginal swabs a significant improvement of isolation rate was found on the samples processed by WASP: its quality of streaking is highly consistent and facilitates plate-reading and subsequent tasks. The machine did not show failures and is very easy to use. WASP full management of the process is faster than operators and guarantees full traceability.

Conclusions: WASP fully processes bacteriology specimens with minimal intervention from operators. The quality and consistency of results using the automation is valuable. Lab technicians have more time to perform other important tasks, generating significant savings.

Identification of clinical isolates of Bacteroides by matrix-assisted laser desorption/ionisation mass spectrometry

E. Urban, M. Kostrewza, T. Maijer, G. Terhes, E. Nagy* on behalf of ESGARAB

Objectives: Members of Bacteroides genus, are important anaerobic pathogens causing severe mixed infections including peritonitis and sepsis. Their correct identification is necessary as resistance to different anti-anaerobic drugs may differ according to the species. As phenotypic identification of anaerobes is difficult, a new approach, the matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-TOF MS) method was evaluated.

Methods: 424 clinical isolates of Bacteroides genus, collected from 8 European countries for antibiotic resistance determination, were identified by MALDI-TOF MS using the Microflex LT instrument and the data processing was performed by the BiotyperTM 2.0 software (Bruker Daltonics, Germany). Mass spectra of each isolate were compared with the mass spectra of 3260 references available. The phenotypic identification for 277 isolates was carried out by classical biochemical tests and by rapid ID32A(ATB) and API20(ANA) (BioMerieux) and was used as reference. 16S rRNA gene sequencing was carried out for a selection of the strains which gave discrepant results and for all those which gave inconclusive identification with the MALDI-TOF. The spectra of sequenced species missing from the data base were added and used for the further identification. For 147 isolates the phenotypic identification was carried out only on the genus level and species identification was carried out by MALDI-TOF.

Results: During the first part of the study out of 277 isolates 270 (97.5%) were unequivocally identified [log(score) ≥ 2.0]. Of the 23 isolates whose MALDI-TOF species identification differed from the phenotypic identification, 11 were sequenced. The sequencing data confirmed the MALDI-TOF result in 10 cases, for one isolate the sequencing did not lead to species determination. Sequencing those 7 isolates, which gave inconclusive identification with MALDI-TOF, revealed species missing from the present data base, such as P. distasonis, and P. goldsteinii. In the second part of the study 147 Bacteroides isolates were identified blindly.
by MALDI-TOF. 145 could be identified on species level using the extended database. There were only 2 isolates, which gave inconclusive identification.

**Conclusion:** MALDI-TOF MS represents a promising tool for rapid identification of Bacteroides strains including newly recognised species and the discriminatory power and identification accuracy of its proved superior to biochemical testing for B. thetaiotaomicron, B. ovatus and B. uniformis.

**Identification of members of the Bacteroides fragilis group by mass-spectrometric discrimination**


**Objectives:** A major part of the human colonic flora consists of the Bacteroides fragilis group. Members of the *B. fragilis* group, in particular *B. fragilis*, are frequently involved in anaerobic or mixed aerobic and anaerobic infections, such as intra-abdominal, gynaecological, and bloodstream infections. These infections are responsible for high rates of morbidity and mortality. The aim of the present study was to evaluate the use of matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) to identify members of the *B. fragilis* group.

**Methods:** A total of 78 cultivated strains of the *B. fragilis* group (*B. caccae* n=9, *B. distasonis* n=5, *B. eggerthii* n=2, *B. fragilis* n=10, *B. melaninogenicus* n=1, *B. ovatus* n=10, *B. stercoris* n=10, *B. thetaiotaomicron* n=10, *B. uniformis* n=11, and *B. vulgatus* n=10), including reference strains and clinical isolates, were analyzed by MALDI-TOF-MS in combination with methods of multivariate statistical analysis. The strains were identified previously by biochemical reactions.

**Results:** The MALDI-TOF-MS analysis was able to discriminate rapidly between the different members of the *B. fragilis* group and showed identical and typical patterns on species level.

**Conclusion:** The results of the present study showed that MALDI-TOF-MS might be used as a rapid method for identification of cultivated strains of the *B. fragilis* group. Thus, the MALDI-TOF-MS can serve as a valuable tool for laboratory diagnosis of infections due to strains of the *B. fragilis* group.

**Matrix-assisted laser desorption ionisation time of flight mass spectrometry is superior to biochemical identification in clinically important Staphylococcus species**

*F. Szabados*, M. Kaase, S. Gutermann (Bochum, DE)

**Objectives:** Recently Matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) was introduced as a new method for bacterial differentiation. The bacterial proteome in the range of 2 kDa to 20 kDa was measured and compared to a database (Biotyper 2.0, Bruker). This method is straightforward, faster and cheaper compared to other methods of bacterial differentiation. Nevertheless, the database for the protein-profiles was not validated sufficiently, only little data was published concerning clinical important strains. The aim of this study was to evaluate the value of this new differentiation-method, compared to the automated biochemical differentiation (Vitek2-system, Biomerieux).

*Staphylococcus lugdunensis* is an uncommon species of coagulase-negative *Staphylococcus* (CoNS) and can cause serious invasive infections such as osteomyelitis and infective endocarditis just as *S. aureus*. Biochemical identification of *Staphylococcus lugdunensis* is challenging, because some strains morphologically resemble *S. aureus*, and further tests are required (e.g. ornithin decarboxylase [ODC], pyrrolidonyl arylamidase [PYR]).

**Methods:** The species differentiation of 215 clinical isolates of *S. aureus* (70 methicillin susceptible and 105 methicillin resistant, 39 methicillin susceptible and pantone valentine leukocidin toxine (PVL) positive *S. aureus* isolates) and 110 clinical isolates of *S. lugdunensis* were re-examined with MALDI-TOF-MS and results were compared to a combined gold-standard (automated biochemical differentiation (Vitek2-system bioMerieux) and sodA sequencing for equivocal results).

**Results:** Bacterial differentiation of clinical important staphylococcus isolates using MALDI-TOF MS is not only superior to biochemical differentiation regarding analytical specificity, but also time to result was reduced significantly.

**Conclusion:** Application of MALDI-TOF for microbial identification and comparison with standard biochemical and molecular identification in a routine laboratory

**Methods:** Ten clinical isolates were selected to test different sample preparation methods: direct application, ethanol (75%) fixation or extraction (ethanol, formic acid, acetonitrile). Influences of various culture media (blood-, chocolate-, CLED- and Sabouraud’s agar) and incubation conditions (time, O2 or CO2 concentrations and temperature) were tested. Thirdly, 189 clinical isolates (30 different genera and 74 species) cultured at regular base in our laboratory, were identified by both MALDI-TOF (duplicates) and routine identification methods such as VITEK-II, API and standard biochemical tests. Discrepancies were further analyzed by molecular sequencing of 16S genes.

**Results:** Gram-negatives (n=5) were correctly identified irrespective of sample preparation method used, Gram-positives (n=4) required direct or extraction method and were poorly identified by ethanol fixation, and yeast (n=1) required extraction. Culture medium as well as overnight, 4–8, 48 or 72 hours of incubation had no influence on accuracy of identification. There was no influence of incubation with O2 or CO2 nor of incubation temperature (4, 30 or 35 degrees Celsius). Of 189 clinical isolates, 179 (94.7%) were correctly identified to the genus level by MALDI-TOF. Furthermore, 152 isolates (80.4%) had correct species identification. Four isolates (2.1%) could not be identified by MALDI-TOF and six isolates (3.2%) had no uniform species identification between duplicates (* Klebsiella planticola * or * K. oxytoca*). There were no incorrect identifications to the genus level. Nine (4.8%) had correct genus identification, but were misidentified by MALDI-TOF with a genetically related species (*Citrobacter koseri* or * C. murliniae*), as determined by 16S analysis. Gram-positive cocci were most difficult to identify to species level with only 51 (69.9%) of 73 correct species identifications. Yeast yielded most correct species (n=7) identifications: 18 out of 19 (94.7%) strains.

**Conclusion:** MALDI-TOF MS can be applied easily without need for special sample preparations, specific culture medium or incubation conditions. Microbial identification by MALDI-TOF has high accuracy to genus level and acceptable accuracy to species level. Incorrect identifications were rare and occurred only at the level of closely related species, not at genus level.

**The performance of matrix-assisted laser desorption/ ionisation time of flight mass spectrometry in the identification of Enterococcus faecalis and E. faecium in clinical isolates**

*G.H. Genzel*, R. Schaumann, W. Schellenberger, A.C. Rodloff, K. Eschrich (Leipzig, DE)

**Objectives:** Enterococci are part of the human physiological intestinal flora, but gained an increasing relevance as a pathogen. Enterococcus faecalis or *E. faecium*, the two species most frequently associated with
human infections, demand a rapid identification and could serve as a first hint towards an effective antibiotic therapy. We investigated the ability of MALDI-TOF MS to distinguish the two species.

**Methods:** To create a reference mass spectra database, 10 strains of *E. faecalis* and *E. faecium* including reference strains and clinical isolates were used, respectively. The strains were identified previously as *E. faecalis* and *E. faecium* by biochemical reactions. MICs were determined by broth microdilution test. Subsequently, all strains were analyzed by MALDI-TOF-MS. Peak lists derived from the mass spectra were analyzed by different methods of multivariate statistical analysis. For classification of *E. faecalis* and *E. faecium* support vector machine algorithms turned out to be most powerful. Thereafter, clinical isolates of *E. faecalis* (n=72) and *E. faecium* (n=28) were analyzed by the blindfolded MALDI-TOF-MS investigator.

**Results:** A reliable reference spectra database for the following investigations was established with 10 strains of *E. faecalis* and *E. faecium*, respectively. From the 100 blindfolded tested strains, the system was able to distinguish 98 strains correctly, only 2 were misidentified. All results were reproducible.

**Conclusion:** MALDI-TOF-MS has an excellent ability to distinguish rapidly between *E. faecalis* and *E. faecium* and can therefore serve as a valuable tool for laboratory diagnosis of Enterococci caused infection.

**P884 MALDI-TOF MS-based identification versus biochemical test systems: a representative study with clinical Enterobacteriaceae isolates**

S. Engels-Schwarzlose, S. Burak*, M. Erhard, I. Streit, A. Gehrt (Düsseldorf, Potsdam, DE)

**Objectives:** The identification of pathogenic microorganisms in a clinical laboratory is commonly based on species-specific biochemical growth and reaction patterns. In contrast, Matrix-Assisted Laser Desorption Ionisation/Time-of-Flight Mass Spectrometry (MALDI-TOF MS) offers a new and promising approach to rapid identification. To establish this method in a routine laboratory, an evaluation study focused on Enterobacteriaceae was performed. We compared the biochemical identification results of clinical Enterobacteriaceae isolates with the results obtained by MALDI-TOF MS.

**Methods:** 816 Enterobacteriaceae isolates with at least 90% probability of biochemical identification by Phoenix (BD, Germany) or Micronaut E (Merlin, Germany) were analysed by MALDI-TOF MS. Small amounts of intact cells of a single colony were directly applied onto the target plate, mixed with matrix solution and air dried. Mass spectra were acquired using an AXIMA-LNR mass spectrometer (Shimadzu, Germany) and analysed by the SARAMIS database tool (AnanosTec, Germany). In case of divergent results, biochemical and MS identification were repeated. If divergence continued, 16S rRNA sequencing was performed.

**Results:** Concordant results of both identification systems were obtained with 686 strains (84.1%). 46 strains (5.6%) had divergent results of MALDI-TOF MS and biochemical identification. Further experiments showed false biochemical identification of 45 strains in the first analysis and still eight in the second one. Only one strain was misidentified in the first and second MS analysis. 10.3% (4.9%) of the isolates could not be identified in the first (second) analysis by MALDI-TOF MS. The discrepancies between the first and the second experiment seem to be correlated with handling inaccuracies of both methods.

**Conclusion:** The great advantage of MALDI-TOF MS/SARAMIS identification of Enterobacteriaceae is the high validity with an error ratio of only 0.12%. The proven robustness of the new method offers the opportunity to facilitate the identification of Enterobacteriaceae in routine laboratories with high sample throughputs. The only incorrect result in this study was corrected by database revision. Due to further development of the SARAMIS database we expect significantly higher identification ratios in the future. MALDI-TOF MS/SARAMIS is a new and easily manageable technique of identification of Enterobacteriaceae with very high reliability.

**P885 Development of an analytical method based on protein profiling for the rapid identification of Aspergillus spp. from clinical samples by MALDI-TOF-MS Biotyper**

L. Patigiani°, L. Mancinelli, L. Dimiziani, C. Russo, L. Colletta, T. Maier, D. Menichella (Rome, Macerata, IT; Leipzig, DE)

**Objectives:** Invasive mould infections are becoming more frequent, resulting in significant morbidity and mortality in children. Paediatric populations are currently at high risk for fungal opportunistic infections due to the high impact of changes in medical practice, intensive care and organ transplantation practises. In our study, we enlarged the library of the MALDI-ToF MS (Matrix-assisted laser description ionisation-time of flight-mass spectrometry) Biotyper with the aim to exploit proteome profiling of Aspergillus spp. to provide an advanced and reliable method for the identification of Aspergillus species from clinical specimens.

**Methods:** Reference and clinical strains of ten *Aspergillus* species were both purchased from the culture collection Centraalbureau voor Schimmelcultures (CBS) and collected in our diagnostic unit to select the most representative species isolated from clinical specimens. Medium growth conditions and protein extraction protocols were optimised to produce suitable template for MALDI-ToF MS analysis. Replicates for each spectrum were collected for each species and analysed for reproducibility. Finally eight overlapping spectra were selected for each species and evaluated for variance by principal component clustering and dendrogram analysis (PCA). Selected spectra were uploaded into the library of the MALDI TOF-MS and added to the pre-existents to perform identification of fungal clinical specimens.

**Results:** The reference spectra of the *Aspergillus* species showed typical MALDI-TOF-MS spectra with peaks between m/z 2000 and up to about m/z 16,000. On visual inspection, the similarity of spectra produced by different *Aspergillus* species could be recognized. Obvious differences between spectra produced by the diverse species were also easily noticed. The reproducibility of the method was proved by the high similarity and PCA outcome of spectra belonging to the same species. Enlarged dataset provided high matching scores (2.3–3.0 range) for *Aspergillus* spp. identification from clinical testing samples.

**Conclusion:** New proteome profiling-based assays for detection of mould fungi may be an optimal diagnostic approach to overcome current culture-based methods, encompass multiple fungal genera, and for being applied to a variety of specimen types. In our experience, MALDI-ToF is currently under setting and may represent a new frontier for the fast and reliable management of fungal infections in paediatric high risk patients.

**P886 Comparison of BioTyper and VITEK2 in identification of bacteria**

E. Kalogeronpoulou°, W. Solbach, J. Knobloch (Lubeck, DE)

**Objective:** MALDI-TOF mass spectrometry (MS) is a simple and fast diagnostic method for the identification and classification of microorganisms. We evaluated the performance of the BioTyper™ system using two different sample preparations (direct smear and Ethanol-formic acid extraction; EFAE) in comparison to the biochemical identification system VITEK2, for bacteria of the clinical microbiology routine.

**Methods:** In a two-month period 1098 aerobicial grown bacterial isolates were obtained from clinical microbiology routine. Strains were cultured on Columbia Blood agar (BioMerieux) and were tested by VITEK2 and the two above mentioned applications of MALDI-TOF MS using the BioTyper™ system in parallel. The results of MS identification were classified as “species identification” (A) “genus identification” (B) or “no reliable identification” (C) as given by the automated BioTyper™ identification system and were compared with the results of VITEK2.

**Results:** 6 (0.5%) were not identified by VITEK2. 32 (2.9%) displayed discrepancy regarding the results of the two methods respectively (PCR-Sequencing will be performed for these 38 isolates). The classification of direct smear/EFAE results (S/E) in A, B and C among the most frequently Gram(+) and Gram(–) isolated species is the following:
**Staphylococcus aureus** (n = 260, 24.5%): A (188/124), B (64/34), C (8/88). Staphylococcus epidermidis (n = 67, 63.5%): A (44/25), B (17/23), C (6/19). Enterococcus faecium (n = 42, 4%): A (31/34), B (4/3), C (7/6). Enterococcus faecalis (n = 33, 3.1%): A (21/22), B (10/6), C (2/5). Escherichia coli (n = 176, 16.6%): A (128/98), B (24/10), C (24/65). Enterobacter cloacae (n = 55, 5.1%): A (40/10), B (8/7), C (7/38). Klebsiella pneumoniae (n = 40, 3.8%): A (29/20), B (4/8), C (9/11). Pseudomonas aeruginosa (n = 99, 9.3%): A (55/45), B (18/21), C (26/33). Acinetobacter baumannii (n = 24, 2.3%): A (11/13), B (3/2), C (10/9). Stenotrophomonas maltophilia (n = 24, 2.3%): A (6/7), B (7/4), C (11/13). Despite the classification by the system all results classified in B displayed the correct species and not only the genus.

**Conclusions:** Discrepancy of bacteria identification between VITEK2 and MALDI-TOF is rare (2.9%). The incidence of no reliable identification regarding direct smear and EFAE preparations of the samples is 15.5% and 31.7% respectively. However, only 3.9% of samples remained without identification by applying both direct smear and EFAE. Modification of the software will result in a higher number of correct species identification.

**New automated solution for plate streaking: comparative evaluation of the PREVI Isola in a microbiology lab**

G. Funke, T. Kemert, B. Barth, C. Fulchiron, G. Bossy* (Ravensburg, DE; La Balme-les-Grottes, FR)

**Objectives:** The study was performed in our routine clinical microbiology laboratory to evaluate the PREVI Isola system in terms of time saving and quality of the results compared to the routine manual method.

**Methods:** A total of 536 urine specimens, 385 faecal specimens, 137 wound swabs, 48 ENT swabs were included in the present study. These samples were processed with the PREVI Isola and the current manual method used in our laboratory. The PREVI Isola is a new automated system able to perform the inoculation and streaking of Prepped Media (PPM) with liquid microbiological samples. According to the specimen type and data previously introduced in the system, the instrument selects the media to inoculate, applies a radial inoculum onto the agar plate, and performs circular streaking with a disposable applicator.

The quality of the automated streaking (AS) was described as lower, equivalent or higher to the manual streaking using two criteria:

a. Isolates must reflect the specimen's microbiological status (polymicrobial sample, presence of pathogen).

b. Isolates must present enough isolated colonies to allow the required identification and susceptibility tests to be performed afterwards.

The time measurement was performed by two persons to record all the steps needed from collection to plates streaking for the management of urine samples for both methods.

**Results:** For urine samples, regardless of the number of isolated, further processable colonies, the PREVI Isola was superior to the manual method.

For faeces, the PREVI Isola was more sensitive than the manual method by detecting 120 isolated colonies vs. 98 on Leifson agar and 77 vs. 66 on SS-agar, respectively.

For wound swabs, the PREVI Isola allowed a faster processing of the cultured material than the manual method.

For both totally independently processed wound and ENT swabs, the PREVI Isola was superior to the manual method in a relatively narrow range from 54.5% to 66.7% for the main growth media and allowed a faster processing of the cultured material than manual method.

The time measurement showed a saving time of up to 57% with PREVI Isola compared to the manual method for the streaking of urines.

**Conclusion:** The PREVI Isola system seems to produce a higher number of colonies suitable for further characterisation than the manual streaking method. Further evaluations in clinical laboratories with larger numbers of clinical specimens in order to prove the suitability of this new system are needed.

**Usefulness and reproducibility of sputum Gram stain in LRTIs**


**Objectives:** Sputum Gram stain examination is a controversial rapid diagnostic method for the presumptive identification of pathogens causing lower respiratory tract infections (LRTIs). The purpose of this study was to evaluate the usefulness and reproducibility of sputum Gram stain for diagnosis of community and hospital-acquired LRTIs.

**Methods:** One thousand eight hundred adult patients (1148 males, 652 females, mean age 64) hospitalised for pneumonia at Sotiria General Hospital, Athens, Greece, were enrolled in the study. Two hundred fifty coded slides were randomly selected and prepared after sputum homogenisation and Gram stain, and three experienced microbiologists evaluated sputum quality. Samples of good quality were assessed for a predominant morphotype. The sensitivity, specificity, positive and negative predictive values of Gram stain compared to culture was calculated. Intra-observer variation was assessed using the kappa statistic (k).

**Results:** One hundred seventy slides (68%) were of good quality and 150 (60%) showed a predominant morphotype. The calculated sensitivity, specificity, positive and negative predictive value were very similar between different observers; for Gram-positive cocci 64%, 42%, 41%, 64%, respectively; and for Gram-negative bacilli 44%, 78%, 87%, 30%, respectively. The sensitivity and specificity of the Gram-positive diplococci identification in the sputum culture of *S. pneumoniae* were 35% and 96%, respectively, and the sensitivity and specificity of the Gram-negative cocacobacilli identification in the sputum culture of *H. influenzae* were 16% and 96%, respectively. Inter-observer agreement for the identification of infected samples was good (k=0.65–0.75 for squamous epithelial cells; k= 0.50–0.60 for neutrophils). Prediction of Gram positive cocci infection showed the greatest agreement between observers (k=0.45–0.85).

**Conclusion:** Sputum Gram stain has been proven useful in guiding microbiological diagnosis of pneumonia in 60% of cases. Specificity of Gram-negative bacilli, Gram-positive diplococci, and Gram-negative cocacobacilli identification was very high. Sputum Gram slide interpretation was reproducible and predictive of a positive culture.

**Performance of the PREVI™ Colour Gram automated staining system**

H. de Montclos*, J. Verdiel (Bourg-en-Bresse, FR)

Gram stain is a critical stage of the microbiological diagnosis. Even though it is usually carried out manually, automation has become an alternative. Bacteriologists used to the manual method may, however, consider that automation allows to subtly adapt the decolourisation step to the thickness of the smear.

**Objectives:** A study was performed to evaluate the performance of the PREVI™ Color Gram system on pure strains and biological samples in comparison with the manual staining method. This new system ensures an automatic Gram staining of slides disposed on a carousel. Dyes are sprayed on slides during rotation.

**Methods:** Manual and automatic stainings were carried out in parallel. Manual Gram stain was performed according to the conventional procedure. The Gram staining on PREVI Color Gram system was performed using the Decolorizer 2 and 3 programs. Slides were then mixed and read by a trained bacteriologist not knowing the bacterial identity and the staining type carried out on each slide. The first part of the study consisted of the staining of 40 reference strains. Results were considered to be concordant when morphology, arrangement and Gram reaction of bacteria were identical. The second part of the study included 107 clinical specimens from various origins. Smears were randomly attributed to the manual or the 2 automated protocols. Preparations were considered to be concordant when the main bacterial populations were found in equivalent proportions.
Results: 321 preparations were compared, 8 of them were declared non-concordant, but not significant as real discrepancies. These discrepancies involved 4 samples and were due to: a. insufficient decolourisation by the manual technique, b. washing out of the smear during manual staining, c. difference in thickness of the smears in the case of a very mucoid expectoration, and d. staining problem which could not be allocated to one or the other of the techniques.

The 2 automated protocols gave the same results. The PREVI Isola system for automated streaking of patient correlation of Sedimax® (Menarini) versus reverse light staining problem which could not be allocated to one or the other of the techniques.

Methods:

E.A. E. Verhoef-Verhage*, A.P.J. van Boxtel, M. van de Wijdeven (Utrecht, NL)

Introduction: The aim of this study was to assess the value of the PREVI Isola (bioMerieux) in the routine diagnostic laboratory, combined with a preliminary cost-benefit analysis. This system uses a fully automated technology that is able to process any material from patients (liquid format) automatically. Different tube sizes can be used to collect patient’s material and they can be loaded into the PREVI Isola. Disposable tips and applicators inoculate different agar plates automatically. A touch screen interfaces with a software program that contains the information which plates should be inoculated for each clinical sample. The PREVI Isola was introduced in our laboratory in December 2008 (Saltro) and we decided to evaluate the technique by first studying urine samples.

Methods: From 100 different patients with urinary tract infections, urine samples (with a positive native slide) were processed manually and by the PREVI Isola.

Results: After overnight incubation, all samples could be evaluated. No significant differences in bacterial count between both methods could be detected. With PREVI Isola, individual colonies were easier to distinguish than after manual inoculation. In general, colonies (especially Proteus spp.) could be easier differentiated than after manual processing. This new system is labour saving: only the samples need to be placed in the machine. Inoculation and streaking are done automatically. We estimated that for every 100 urine samples 1 h of manual work could be saved.

Conclusions: PREVI Isola is a new technology that leads to better readable results for urine samples. It saves labour time and therefore costs. Because individual colonies were more frequently observed, sub culturing and therefore identification and susceptibility testing can in some cases be done sooner. Also by introducing elective agar plates, identification could be done 24 hrs earlier. Further studies are needed with different materials to obtain a definitive analysis of this method.

Method: Routine urine examination is done by RLM after sedimentation of 60 μl of sample in a multwell plate. Leucocytes and erythrocytes are quantified. Bacteria, cellular and other elements are identified qualitatively. Quantitative culture is performed on a Cystine-Lactose-Electrolyte-Deficient (CLED) plate. The Sedimax® was used according to the instructions of the manufacturer. Both methods were compared on 782 consecutive routine samples during a 3 weeks period. All samples were examined within 30 minutes by the two methods. Results for leucocytes and erythrocytes were divided into classes based on their number. A difference of one class was defined as a minor deviation, a difference of more than one class as a major deviation. A positive significant culture of >100 CFU/ml was used as gold standard to evaluate the Sedimax® and RLM interpretation of bacteruria.

Results: 698/782 (89.3%) urine specimens could be evaluated. Due to some technical problems 4.1% of the samples were not examined correctly by the Sedimax® and 7.5% of samples did not contain enough bacteria. Concordance for leuco- and erythrocytes between both methods was respectively 73.1 and 82.8%; 26.9% of the urine samples showed major discordances for white blood cells and 17.2% for red blood cells. In most cases this is due to misinterpretation by the Sedimax® of other elements present in the urine such as clumped cells, amorph sediment, crystals, epithelial cells. Using the manufacturer’s criteria for bacteruria, concordance between the two methods was almost 73%. The Sedimax® produced 4.3% false negative results and 26.1% false positives. This may be caused by a too low cut off value for bacteria in the Sedimax®. Instead the RLM reports only 5.9% false negative and 4% false positive results.

Conclusion: In its current version, Sedimax® is not superior to our RLM method in workload and hands on time. Given the high number of discordance of white and red blood cells, the Sedimax® can not be used in our laboratory without further adjustments.

Correlation of Sedimax® (Menarini) versus reverse light microscopy for the examination of urine sediment

H. Devos, I. Mermans, H. Goossens, M. Ieuen* (Edegem, BE)

Objective: Automated analysis of insoluble urine components can reduce the workload and hands on time of conventional microscopic examination of urine sediment. Urinary flow cytometry and automated microscopic pattern recognition are two new techniques. The objective of this study was to compare the reversed light microscopy (RLM) method with the Sedimax® (Menarini), a fully automated urine analyser.

Method: Routine urine examination is done by RLM after sedimentation of 60 μl of sample in a multiwell plate. Leucocytes and erythrocytes are quantified. Bacteria, cellular and other elements are identified qualitatively. Quantitative culture is performed on a Cystine-Lactose-Electrolyte-Deficient (CLED) plate. The Sedimax® was used according to the instructions of the manufacturer. Both methods were compared on 782 consecutive routine samples during a 3 weeks period. All samples were examined within 30 minutes by the two methods. Results for leucocytes and erythrocytes were divided into classes based on their number. A difference of one class was defined as a minor deviation, a difference of more than one class as a major deviation. A positive significant culture of >100 CFU/ml was used as gold standard to evaluate the Sedimax® and RLM interpretation of bacteruria.

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Diagnostic methods

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In vitro effects of iodinated contrast agents on bacterial growth


Objectives: Injection of iodinated arthrographic contrast medium in (artificial) joints (puncture arthrography) is used to diagnose infection or aseptic loosening of arthroplastics. During this procedure synovial fluid is aspirated and submitted to the microbiology laboratory for culture. Inevitably, contrast medium will be mixed into the sample. The fact that iodine is used as a disinfectant has led to the assumption that this will adversely affect culture results. Despite previous reports on contrast media inhibiting bacterial growth, the role of iodine herein is questionable. Contrary to free iodine, iodine in a bound state is not bactericidal. Moreover, currently used media are iso-osmolar. In this study, we assessed in vitro bacteriostatic and bactericidal properties of iodinated contrast media.

Methods: The influence of three currently used media (Omnipaque®, Visipaque®, Xenetix®) and two older media (Hexabrix®, Telebrix®) on the growth of pathogens commonly isolated in prosthetic infections was studied using well-defined strains of Bacillus cereus, Candida albicans (C. albicans), Corynebacterium jeikeium, Enterococcus faecalis (E. faecalis), Escherichia coli (E. coli), Propionibacterium acnes, Pseudomonas aeroginosa (P. aeroginosa), Staphylococcus aureus, Staphylococcus epidermidis and Stre!tococcus pyogenes. Inhibition was determined using a disk diffusion technique and a time-killing curve method in which high (5×10⁸ CFU/ml) and low (5×10⁷ CFU/ml) inocula were tested respectively. Colony counts were performed of freshly prepared suspensions in contrast medium and a saline control and after 2 and 24 hours of incubation at 35°C.

Results: With disk diffusion testing we found no influence on the growth of micro-organisms of any of the media. In the high inoculum tests the only, non-significant, growth inhibition occurred with the combination of Telebrix® and E. coli (1 log10, P = 0.07). In the low inoculum tests
both *E. coli* and *P. aeruginosa* were inhibited by Telebrix® but not by any other medium. For all other combinations there was no inhibition compared to the saline control, except for some media and *E. faecalis* and *C. albicans*. In these cases however, the percentage surviving cells was always higher than 30%.

**Conclusion:** The effect of the dated medium Telebrix® on *E. coli* and *P. aeruginosa* seems consistent with previous reports. There is no evidence that currently used iodinated contrast media impede detection of micro-organisms in synovial fluid.

**P983** Fever of unknown origin: differential diagnosis between infectious and non-infectious causes

**Objective:** The aims of the present study were (a) to develop a simple and reliable diagnostic model that could aid physicians to discriminate between infectious and non-infectious causes of fever of unknown origin (FUO), and (b) to evaluate the performance of the derived tool in an independent database of subjects with FUO.

**Methods:** Participants were patients with classical FUO fulfilling the modified criteria of Durack and Street (from 1992 to 2000) and an external validation study (from 2001 to 2007). In the internal phase, 33 variables regarding each patient's demographic characteristics, history, symptoms, signs, and laboratory profile were recorded and considered in a logistic regression analysis using the diagnosis of infection as dependent variable. In the external phase, the model derived on the basis of the independent predictors of diagnosis of infection was applied on the next consecutive subjects with FUO and the respective discriminatory capacity was calculated.

**Results:** Data from 112 hospitalised individuals (mean age 56±11.2 years, 55% males, fever duration before admission 32 ±11.9 days) were analyzed in the internal study. The final diagnoses included infections, malignancies, non-infectious inflammatory diseases, and miscellaneous conditions in 30.4%, 10.7%, 33% and 5.4% of subjects, whereas 20.5% of cases remained undiagnosed. C-reactive protein >60 mg/L (odds ratio 6.0 [95% confidence intervals 2.5, 9.8]), eosinophils <40/mm³ (4.1 [2.0, 7.3]) and ferritin <500 μg/L (2.5 [1.3, 5.2]) were independently associated with diagnosis of infection. Among the 100 patients of the external study, the presence of ≥ 2 of the above factors predicted infection with sensitivity, specificity, and positive and negative predictive values of 91.4%, 92.3%, 86.5%, and 95.2%, respectively. Thus, the overall discriminatory capacity of the model – when the cut-off of ≥2 factors was used – corresponded to an area under the curve (AUC) of 0.92 (95% CI 0.85, 0.98; p <0.001), whereas the respective AUC values of its three components were 0.75 for C-reactive protein [95% CI 0.65, 0.86], 0.70 for eosinopenia [0.59, 0.80], and 0.68 for ferritin [0.57, 0.78].

**Conclusions:** The combination of C-reactive protein, ferritin and eosinophil count may be useful in discriminating infectious from non-infectious causes in patients hospitalised for classical FUO.

**P984** The diagnostic accuracy of endotracheal aspiration and bronchoalveolar lavage in the diagnosis of ventilator-associated pneumonia
H. Gedik*, M. Yahyaoglu, M. Fincanci (Istanbul, TR)

**Objective:** To assess the diagnostic accuracy of endotracheal aspiration (ETA) and mini-non-bronchoscopic, protected bronchial lavage (mini-BAL) in the established clinical diagnosis of VAP.

**Design:** Prospective observational study using ETA and mini-BAL collected within 24h.

**Setting:** This prospective study was conducted in a 11adult-bed surgical ICU between August 2004 and August 2005.

**Patients:** Thirty-one patients hospitalised for more than 72h, who were mechanically ventilated and had a new or progressive lung infiltrate plus at least two of the three clinical criteria for VAP

**Interventions:** Diagnostic threshold for ETA was considered >100,000 cfu/ml and for mini-BAL >1000 cfu/ml.

**Results:** Quantitative cultures of ETA and mini-BAL cultures yielded negative results for 23.2% of patients with clinically suspected VAP. The agreement between the microbiological results obtained from the two techniques was assessed according to the statistical methods (p: 0.005, kappa: 0.804). Existence of leukocytes and bacteria in ETA samples were correlated with culture results (p: 0.001; kappa: 0.395) and in mini-BAL samples, as well (p: 0.003, kappa: 0.460).

**Conclusions:** Clinical and radiological diagnosis of VAP without microbiological cultures may be misleading. Quantitative cultures of ETA and mini-BAL were in agreement in our study. Also quantitative culture of ETA may be adequate for routine diagnosis of VAP Gram staining of ETA and mini-BAL samples could provide important clues for early and appropriate antimicrobial treatment.

**P985** Utility of alveolar and ascitic sTREM-1 to diagnose aetiology of acute respiratory distress syndrome

Critical care patients with abdominal pathologies often developed an acute respiratory distress syndrome (ARDS). ARDS can be due to the abdominal pathology or can be caused by a nosocomial pulmonary infection. Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) has shown to be of utility in diagnosing pulmonary infections. Our objective was to assess the utility of sTREM-1 in the aetiological diagnosis of ARDS in critical care patients with abdominal pathology.

**Methods:** On the first 4 days of ARDS we analysed alveolar and peritoneal fluid: microbiological and cytological analysis and sTREM-1 by an ELISA method. Clinical and laboratory tests were performed to complete diagnosis. Statistical analysis included U-Mann-Whitney test.

**Results:** We included 21 patients. Mean age was 48 years (DE 17) and 68% were men. Mean APACHE II was 19 points (DE 6) on ICU admission day and SOFA value on the day on evaluation was 13 points (DE 3). The most frequent abdominal pathologies were: spontaneous bacterial peritonitis (27%), enteritis (23%) and pancreatitis (14%). In 41% of the patients a pulmonary infection was diagnosed, in 27% an abdominal infection was diagnosed, in 27% both infections coexisted and in 5% no focus could be identified. Mean alveolar sTREM-1 was 1713 pg/ml, 1512 pg/ml if an abdominal infection existed and 2027 pg/ml if a pulmonary infection was diagnosed. Mean peritoneal sTREM-1 was 1424 pg/ml, 2198 pg/ml if an abdominal infection existed and 1275 pg/ml if a pulmonary infection was diagnosed. To distinguish between the absence and presence of an abdominal infection peritoneal sTREM-1 (<0.001) and serum procalcitonin (p 0.18) were of utility. To distinguish between the absence and presence of a pulmonary infection alveolar sTREM-1 (p 0.019) was of utility. A ratio alveolar sTREM-1/peritoneal sTREM-1 >1 strongly indicated a pulmonary infection (sensitivity 63%, specificity 100%) whereas a ratio <2 strongly indicated an abdominal infection (sensitivity 99%, specificity 83%).

**Conclusions:** In a critical care patient the compartmentalisation of the inflammatory response in the initial infectious focus is not the regular performance. However, sTREM-1 keeps certain capacity to indicate the infectious origin. To establish the aetiology of ARDS could have important treatment implications, meanly the indication of surgery if an abdominal infection is diagnosed.

**P986** Procalcitonin in abdominal surgery: is there an economical interest?
F. Meurant, P. Geukens° (Luxembourg, LU)

**Objectives:** Septis and severe infections are common causes of morbidity and mortality in intensive care units. The abuse of antibiotics is known to promote pathogens resistance and increase long term morbidity. Procalcitonine (PCT) could indicate the right time to introduce
antibiotics in patients but is still an expensive laboratory test. We attempt to evaluate benefits of using this biological marker and its economical interest in daily antibiotic therapy.

**Methods:** 66 patients who had an elective non-complicated cholecystectomy and who developed criteria of systemic inflammatory response (Temperature >38°C or <36°C, WBC count >12,000/mm³ or <4000/mm³, or >10% immature (band)), without positive bacterial culture, were randomised prospectively in our ICU directly after the surgical procedure. Each patient received one shot of Amoxicillin–Clavulanic Acid (2 g) during surgery. In the control group (Gs: n = 33) the antibiotic therapy was stopped right after the surgery, compared to the study group (Gs: n = 33) where the antibiotics were given during 2 days. Serum PCT levels were measured each day in the two groups. Samples of blood, mid stream and lung expectoration cultures were also taken every day. Antibiotic costs, were compared between groups. For statistical analysis a Shapiro–Wilk test, Wilcon and a student T-test were used.

**Results:** Demographic data were comparable in terms of gender, age, SAPS and operating time (mean 1 h25+/-15 min). During the first 2 days in the Gc 8 patients developed infections compared to 7 in the Gs (p<0.05). For 6 patients in Gc, a bacterial identification allowed to guide a cheaper antibiotic choice compared to the Gs (p<0.01) where empirical antibiotic therapy appeared to be more expensive.

**Conclusion:** It is not the systematical prolonged antibiotic therapy after elective surgery who can protect against infections. This attitude avoids the benefit of bacterial identification and so far has an economical bad impact on the choice of antibiotic therapy. PCT could help to choose which patient would benefit from prolonged post-surgery antibiotics.

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**Plasma procalcitonin levels in clinically stable patients with cystic fibrosis**

*AP Watt*, J. Courtney, J.S. Elborn (Belfast, UK)

**Objective:** Chronic bacterial infection is a major cause of morbidity and mortality in Cystic Fibrosis (CF). The identification of inflammatory biomarkers which correlate with lung injury and infection may be useful in monitoring progressive pulmonary disease. Blood procalcitonin (PCT) levels are raised in bacterial infections and remain low in viral infections and non-specific inflammatory diseases. To date, no studies have been published evaluating PCT levels in CF: the objective of this study is to establish baseline plasma PCT and examine the possible role of PCT as a biomarker useful in rationalisation of antibiotic therapy in CF.

**Methods:** BRAHMS PCT immunoluminometric kit was used to measure PCT in 163 retrospective anonymised plasma samples. 85 plasma samples were from patients with CF who had no history in the previous four weeks of hospitalisation for an acute exacerbation and/or intravenous antibiotics. 78 samples were from non CF individuals. Retrospective data on C-reactive protein (CRP) and alpha 1 antitrypsin for the two groups studied were compared using the non-parametric Mann Whitney U test.

**Results:** No significant difference in the levels of plasma PCT were observed between the two groups – CF samples (Mean±SEM, 0.14±0.05 ng/ml), and 78 control samples (0.16±0.01 ng/ml). AAT was significantly higher in patients with CF (1.69±0.05 g/L) compared to Controls (1.38±0.03 g/L, p<0.001), whilst CRP was significantly higher in the CF group (13.02±1.39 mg/L) compared to controls (5.4±0.45 mg/L, p<0.001). No correlation was observed between PCT and CRP or AAT. No significant difference in PCT levels was observed between those patients with CF infected with *P. aeruginosa* (n=47, 0.16 ng/ml) and those infected with *B. cepacia* (n=18, 0.16 ng/ml).

**Conclusions:** This pilot study reports no significant difference in the levels of plasma PCT in clinically stable patients with CF compared to healthy controls even when CRP and AAT are significantly elevated. The low levels of PCT observed may reflect on the compartmentalisation of infection within the lungs. Further work is required to investigate PCT levels during pulmonary exacerbations.

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**Changes in platelet sizes as indicators for clinical outcome of bacteremia**

T. Kitazawa*, Y. Yoshino, K. Tatsumo, Y. Ota, K. Koike (Tokyo, JP)

**Objectives:** Systemic infections such as bacteremia induce acute phase responses, leading to multiple alterations including the haemostatic system. It has been reported in many studies that thrombocytopenia is observed during sepsis. However, little is known about changes in platelet sizes in the course of sepsis and association between the changes and the outcome of sepsis. The aim of this study is to elucidate whether the changes or the levels of platelet sizes were associated with the prognosis of bacteremia.

**Methods:** From April 2003 to March 2006, all the patients aged ≥ 20 years whose blood cultures were positive at the University of Tokyo Hospital were enrolled in this study. We selected 350 patients whose platelet volume (MPV) were tested during the three periods: the first period, between 30 days and 7 days before the onset of bacteremia, the second period, ≤ 1 day from the onset, and the third period, 3 to 5 days from the onset. A retrospective chart review was performed to collect demographics. The end point was defined as 30-day mortality.

**Results:** Average MPV at the first period were 7.33±0.90 fl, and increased to 7.66±1.06 fl at the second period and 7.89±1.21 fl at the third period, respectively, with statistical significance (p<0.001). There were no differences in average MPV at the three points between in non-survivors (n=25) and in survivors (n=325). However, the levels of average MPV increase between the first and the second periods were significantly smaller in non-survivors than those in survivors (0.00±0.55 vs 0.35±0.74, p = 0.006). While, those between the second and the third periods were significantly larger in non-survivors than those in survivors (0.74±0.70 vs 0.19±1.18, p = 0.03). Using the changes of MPV in the two intervals, the area under the receiver operating characteristic curves was 0.71.

**Conclusion:** Changes in MPV during early time of bacteremia serve as prognostic indicators for bacteremia.

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**Lymphocytopenia and neutrophil/lymphocyte count ratio predict bacteremia better than conventional parameters in an emergency care unit**


**Objectives:** Prediction of bacteremia is essential in clinical decision-making. Absolute lymphocytopenia (lymphocyte count <1.0×10⁹/L) has been reported as predictor of bacteremia in medical emergencies. Likewise, the ratio of neutrophil and lymphocyte counts (NLCR, normal value 0.4–7.5) has been shown a simple promising method to evaluate the extent of stress or systemic inflammation in critically ill patients. We studied the association between several routine chemical and haematological infection markers and bacteremia in patients presenting to the emergency care unit of the Jeroen Bosch Hospital, an 800-bed teaching hospital in the Netherlands.

**Methods:** During a seven month period one ore more blood cultures were obtained from 746 adult patients. Contaminated blood cultures were excluded (n=29) as well as positive blood cultures from patients with chemotherapy, haematological diseases, glucocorticoids use (n=14) or patients with incomplete data (n=12). C-reactive protein (CRP) level, white blood cell (WBC), neutrophil and lymphocyte counts and the NLCR were compared upon admission between patients with positive blood cultures (n=92, 64% Gram-negative bacteria versus 36% Gram-positive) and age and gender matched control patients with negative blood cultures. SPSS 15 was used to evaluate differences between cases and controls.

**Results:** Paired Student's t-tests revealed significant differences in CRP level, lymphocyte count and NLCR between patients with positive and negative blood cultures. Area under the receiver operating characteristic curve was highest for the NLCR.
Conclusion: In the setting of an emergency care unit, lymphocytopenia and, even more, the NLCR, is more predictive of bacteremia than routine parameters like CRP, WBC or neutrophil count.

Table 1. Predicting bacteremia using conventional parameters (CRP, WBC and neutrophil count), lymphocytopenia and the neutrophil/lymphocyte count ratio

<table>
<thead>
<tr>
<th>Parameter, mean±SD</th>
<th>Cases, n=92</th>
<th>Controls, n=92</th>
<th>ROC area</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>176±138 mg/L</td>
<td>116±103 mg/L</td>
<td>0.62</td>
</tr>
<tr>
<td>WBC count</td>
<td>13.6±6.6 x10^3/L</td>
<td>12.9±5.2 x10^3/L</td>
<td>0.53</td>
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<tr>
<td>Neutrophil count</td>
<td>12.1±6.1 x10^3/L</td>
<td>10.7±5.1 x10^3/L</td>
<td>0.58</td>
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<tr>
<td>Lymphocyte count</td>
<td>0.8±0.5 x10^3/L</td>
<td>1.2±0.7 x10^3/L</td>
<td>0.73</td>
</tr>
<tr>
<td>NLCR</td>
<td>20.9±13.3</td>
<td>13.2±14.1</td>
<td>0.74</td>
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</table>

Fungal diagnostics and pathogenesis

**Development of PCR Electrospray ionisation mass spectrometry for identification of clinically relevant fungal and yeast**


**Background:** Diagnosis of candidaemia typically relies upon blood cultures, which often require several days for correct species identification. Diagnosis of invasive fungal infection (IFI) relies upon a consensus of clinical and laboratory criteria with certainty ranging from definite to probable or possible. Because definite diagnosis requires visualisation of the organism in tissue, the majority of patients with IFI fall into the probable or possible categories. We investigated the hypothesis that a single molecular assay based upon broad PCR amplification followed by mass spectrometry could be used to identify a wide variety of fungi and yeast.

**Methods:** Four broad-range PCR primers were selected within the 5.8S and 26S rRNAs for detection of fungi such that the product base compositions could be used to directly identify species or narrow possibilities to a range of species. Additional twelve primer pairs were designed to identify specific groups by virtue of product amplification and identify species by virtue of product base compositions. Automated PCR/Electrospray ionisation mass spectrometry and data analysis provide base composition signatures that identify clinically-relevant species of fungi and yeast.

**Results:** An assay has been defined to target a range of fungi and yeast that consists of 16 primer pairs, allowing 6 samples to be run on a 96-well plate. The assay broadly detects fungi and yeast, and identifies members of Candida spp. (speciating C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, and C. krusei), Aspergillus spp., Cryptococcus neoformans, Mucorales, Coccioidioides immitis/posadasi, Ajellomyces spp. and, Fusarium spp. A collection of clinically-relevant isolates consisting of 25 Aspergillus spp. isolates, 58 Candida spp. isolates, 13 Cryptococcus spp. isolates, 10 Mucor/Rhizopus isolates, 5 Saccharomyces isolates and 9 Sordariomycete isolates have been tested and correctly identified with automated data analysis. assay limits of detection were determined using quantitated C. albicans and C. glabrata isolates obtained from ATCC. Both species were detected at 30–60 genome copies/PCR.

**Conclusion:** PCR/ESI-MS is a novel approach to fungal diagnostics with potential for rapid identification of a broad variety of fungal or yeast pathogens in a single assay.

**Detection of candidaemia in patients with and without underlying haematological disease**

M.C. Arendrup*, O.J. Bergmann, L. Larsson, H. V. Nielsen, J.O. Jarlon, B. Christensson (Copenhagen, DK; Lund, SE)

**Objectives:** The diagnosis of candidaemia remains difficult despite the development of new diagnostic tools. We here compare the diagnostic potential of three different blood culture systems, D/L arabinofitol, antigen, and antibody detection.

**Methods:** 114 episodes in 93 patients with either underlying haematological disease and fever without response to antibacterial treatment or documented systemic Candida infection were enrolled prospectively. A pro forma was provided to gather clinical and para-clinical information on all patients. Blood culture (BC) was done using a conventional blood culture (2 aerobic and 2 anaerobic bottles), a fungal blood culture (Mycosis bottle, BACTEC), and the Isolator 10 lysis centrifugation blood sample (Isolator 10, Wampole). Surveillance cultures from oropharynx, urine, and faeces were performed. The D/L arabinofitol ratio in serum and urine was determined by gas chromatography (Larsson L, 1994). Antigen (Ag) was detected by the CandTec Kit (Ramco Laboratories Inc., Verrn, Houston, USA) and by using the Plateletia Candida Ag ELISA kit (Bio-Rad, France) and anti-Mannan antibody (Ab) was detected using Plateletia Candida AB/AC/AK kit (Bio-Rad, France).

**Results:** The 114 episodes were classified as proven invasive candidiasis (24), probable (14), possible (52), or unlikely (24). Candidaemia involved C. albicans (17 episodes), C. albicans + C. glabrata (3 episodes), C. tropicalis (1 episode), and yeast not identified (1 episode). The mycosis bottle was the only BC positive in 2 episodes and the conventional blood culture the only BC positive in 1 episode. Evaluating only the proven and unlikely episodes sensitivity (sens) and specificity (spec) of the indirect tests were as follows Mannan Ag & anti-Mannan Ab: 83.3%, 78.3%; D/L arabinofitol ratio in serum: 41.7%, 86.4%; CandTec Candida Ag: 66.6%, 70.8%. By lowering the cut off values to Mannan Ag: 0.10ng/ml and anti-Mannan Ab: 4 AU/ml the values were: sens 100%, spec 73.9%, PPV 80% and NPV 100%, and by applying the D/L arabinofitol ratio to only patients with haematological neutropaenia the values were: sens 75%, spec 90.5%, PPV 60%, and NPV 95%.

**Conclusion:** The fungal blood culture bottle slightly improved the detection of candidaemia. Among the indirect tests the combined use of Mannan Ag and anti-Mannan Ab showed the best performance especially when cut offs were lowered. D/L arabinofitol ratio seemed useful in the setting of haematological underlying disease and neutropaenia.

**Candida mannan and anti-mannan in the diagnosis of invasive candidal infections in neutropenic patients**

M. Ellis*, B. al-Ramadi, R. Bernsen, J. Kristensen, H. Alizadeh, U. Hedstrom (Al Ain, AE)

**Objective:** Antifungal (AF) therapy given to all pts with febrile neutropaenia (FN) is costly and toxic. Beta-D-glucan assay is fungal-specific; galactomannan is useful only for aspergillosis. This study focused on serial assay of mannan (M) and mannan-antibodies (MA) to diagnose invasive candidiasis (IC) in pts with FN, hence to aid selection of pts for AF. Previous experience with this assay has been limited to non-neutropenic pts, based on retrospective infrequent sampling.

**Methods:** 100 patients with acute leukaemia undergoing chemotherapy complicated by FN, given liposomal amphotericin B, were studied prospectively with clinical, microbiological (blood culture), and radiological (CT scans chest, liver, spleen, sinuses) evaluations for development of IC, based on revised EORTC/MSG diagnostic criteria. M + MA were measured daily using Platelia Candida specific antigen/antibody ELISA kits (Bio-Rad). Diagnostic cut-offs were determined using ROC curves.

**Results:** 12 of 86 (14%) eligible pts had IC [ C. albicans candidaemia (1), C. tropicalis candidaemia (4), hematopoeitic candidaemia (7)], 24 had invasive mould, 50 persistent FN. These last 2 groups served as the comparison group. Cut-offs were 0.25 ng/ml and 2.5AU/ml for M and MA, lower than manufacturer's recommendations. All pts with IC developed ≥ 1 +ve diagnostic M or MA during persistent FN (FIG1). Optimal overall performance occurred when 2 consecutive positive tests for both M and MA were used. Sensitivity, specificity, PPV and NPV [95%CI] were 0.73(0.39–0.94), 0.80 [0.69–0.89], 0.36[0.17–0.59], 0.95[0.86–0.99] respectively.
A positive correlation ($r=0.28$, $p=0.01$) was seen with previously determined beta-D-glucan (BDG) concentrations in these pts. The first +ve M test occurred at a mean ±s.d. of 8.8 ±8.5 (2 to 23) days prior to clinical/mycological diagnosis of IC.

High MA concentrations were delayed until leucopenia resolved. The candidal colonisation index (CCI) was $\geq 0.5$ in 60% of the comparison group.

**Conclusions:** Using institution-based cut off values, early serial determination of combined M+MA is useful to diagnose IC in FN pts. Low PPV may reflect low prevalence of IC, whilst the high NPV confidently excludes IC. Negative M+MA tests in the presence of +ve BDG test indicates IC rather than mould. Specificity may be low as a result of subclinical candida infection in the comparator group (high CCI). This assay could be used as part of a broad fungal diagnostic strategy aimed at tailoring AF.

**Results:** At mid-log phase (6h, concentration of cell suspension was $(5.0\pm1.18)\times10^6$ cell/ml), the adherent cell number on FEP and polyurethane catheters were $(8.25\pm0.62)\times10^3$ CFU and $(5.62\pm0.93)\times10^4$ CFU respectively ($p<0.05$). The results of viable count of YPD broth immersing catheters were zero, showing sonication process is complete.

**Conclusion:** FEP catheter and polyurethane catheter have different adhesion features for Candida albicans SC5314 under dynamic environments. Polyurethane catheter has stronger adhesion to Candida albicans SC5314.

**Objectives:** Yeasts are commonly implicated in superficial skin and mucosal infections in the community as well as in invasive infections in immunocompromised and seriously ill patients. Their identification to species level can have consequences for the diagnosis, epidemiology and treatment choice. Various automated systems are commercially available but few have been systematically tested with strains identified by molecular methods. The aim of the study was the evaluation of three main automated yeast identification systems, using for the first time local clinical strains.

**Methods:** 93 clinical isolates (comprising 24 species) were identified by the VITEK 2 system, Auxacolor KAI API ID32C. The results were compared to the reference identification method that employed sequencing of ribosomal regions.

**Results:** From the 61 strains belonging to the clinically most common species C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. lusitaniae and C. krusei, Auxacolor correctly identified 95.1% of the strains, VITEK 2 90.2% and API ID32C 86.9%. From the 32 strains of rare species, the corresponding percentages were 43.8% (Auxacolor), 71.9% (VITEK 2) and 87.5% (API ID32C).

**Conclusion:** Auxacolor seems to be an excellent tool for screening common yeasts in the clinical laboratory. Rapid and fully automated identification was achieved by VITEK 2 (18h versus 48–96h for Auxacolor and API ID32C respectively). Generally, API ID32C and VITEK 2 were equally effective. No system could identify all uncommon, clinically relevant, non-C. albicans species. Consequently, these strains should be polyphasically identified by a combination of biochemical, physiological, morphological and genomic studies.
**Anti-aspergillus antibodies and galactomannan antigen detection for serodiagnosis of aspergillosis**

N. Tsagarakis*, N. Kentrou, V Margarit, A. Malgarinou, S. Maurea, I. Tsagarakis, E. Anastasakou (Athens, GR)

**Objectives:** The development of minimally invasive diagnostic methods is a major advance in the early diagnosis of aspergillosis. Clinically relevant antigens have been adapted for use in immunoassays for the detection of specific antibodies of *Aspergillus fumigatus*, while the detection of circulating galactomannan antigen is a useful tool for serodiagnosis of aspergillosis. The aim of this study was to investigate the concordance of anti-aspergillus antibodies and galactomannan antigen detection in cases suspicious for *Aspergillus fumigatus* infection.

**Methods:** Sera from patients hospitalised in our hospital during the period 1/1/2007 – 1/12/2008 with clinical suspicion of aspergillosis, were examined for detection of anti-Aspergillus antibodies, by SERION ELISA classic *Aspergillus* IgG/IgM/IgA indirect enzyme-linked immunosorbent assays. All positive sera were processed for the detection of galactomannan (GM) by the Platelia Aspergillus ELISA EIA (Bio-Rad), a sandwich immunocapture ELISA, according to manufacturer’s instructions. All patients were investigated for diagnostic purposes, so they were examined for the first time.

**Results:** Among 1288 sera tested, 139 (10.8%) were positive for the detection of IgM antibody, 1070 (83.1%) were negative and 79 (6.1%) were determined to the gray zone. For the detection of IgG and IgA antibody, the results were: 111 (8.6%) and 80 (6.2%) positive, 1119 (86.9%) and 1168 (90.7%) negative, while 58 (4.5%) and 40 (3.1%) belonged to the gray zone, respectively. All anti-Aspergillus antibody positive sera were also tested for the detection of galactomannan (GM), so 35 patients were found positive. Among these patients 28 (80%) were hospitalised in Pneumonological Departments, 4 (11.4%) in Departments of Internal Medicine, and 3 (8.6%) in ICUs. Moreover, 16 (45.7%) patients were simultaneously positive for GM and IgM, 5 (14.3%) for GM, IgG and IgA, 3 (8.6%) for GM, IgG and IgG, 3 (8.6%) for GM, IgG, IgA and IgM. Also, 3 (8.6%) patients were simultaneously positive for GM and IgA only and 2 (5.7%) for GM and IgG.

**Conclusions:** The low concordance of anti-Aspergillus antibodies and galactomannan antigen detection, but also the different types of antibodies simultaneously detected with circulating galactomannan antigen, suggest that the combined examination increase the sensitivity and specificity of both tests, avoiding the cross-reactivity of galactomannan and the false-positive results of antibodies.

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**Evaluation of serum Aspergillus galactomannan in haematological patients**

R. Banon*, R. Rojas, M. Causse, J. Serrano (Cordoba, ES)

**Introduction:** The incidence of invasive aspergillosis (IA) is significantly high in haematological patients. Nevertheless this fungal infection is characterised by a high mortality rate in these high risk patients. An early instituted antifungal therapy should improve the patient prognosis. However, IA is always difficult to diagnose. Major criteria for IA suspicion are essentially based on a set of arguments such as abnormal chest computed tomography scan, and the detection of circulating galactomannan (GM).

**Objective:** Our study evaluated the efficiency for IA diagnosis of the Platelia Aspergillus test routinely performed in a prospective serological survey of haematological patients.

**Material and Methods:** From June, 2002 to June, 2008, 5397 serum samples from 383 patients from haematological department of Córdoba University Hospital that were at risk for IA were tested in a routine screening program, including two blood samples obtained per week. Patients were classified according to IA diagnosis criteria of European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) with three levels of certainty: proven, probable, and possible. Proven IA was difficult to establish because tissue samples were not systematically taken, not even the cultures are requested, and autopsy was never performed. Excluding the galactomannan antigen as microbiological criterion, we have considered 34 patients to be probable or possible IA. The detection of GM by the Platelia Aspergillus test (Bio-Rad, Marnes-la-Coquette, France) was carried out according to the manufacturer’s instructions. We consider as reactive to the GM antigen, that one with two or more positive samples (index 0.5 or major) in the length of one week.

**Results:** Of all the patients studied, only 36 cases were reagents to the GM antigen. The patient’s proportion considered probable or possible IA that obtained a positive result in the diagnostic test was 61.8%, whereas the probability of a patient without IA to get a negative result was 95.7%. The probability of suffering IA if a positive result is obtained in the test was 58.3% and the probability of not suffering IA if a negative result is obtained was 96.3%.

**Conclusion:** Employment of the GM test, in a population of high risk, to detect patients with minor risk of suffering IA, allows to avoid unnecessary treatments in these setting. Consequently we think that it is a useful test for the control of haematologic patients.
**P908** Immune evasion of *Aspergillus fumigatus* by secreted proteases can be undermined by application of amino acids

G. Rambach*, D. Dum, I. Moksenipour, C. Lass-Flörl, R. Würzner, C. Speth (Innsbruck, AT)

**Objectives:** The high lethality of cerebral aspergillosis delivers evidence for efficient immune evasion mechanisms of *Aspergillus*. An important target for evasion mechanisms is the complement system as a key player of innate immunity in the CNS. As our studies indicate that secreted fungal proteases can degrade complement proteins we aim to target these proteolytic enzymes by new therapeutic approaches.

**Methods:** *Aspergillus* spp was grown in medium or cerebrospinal fluid (CSF). Different supplements were used to study their influence on protease secretion. The degradation of soluble immune proteins was investigated by Western Blot, cellular expression of surface proteins was quantified by FACS. Fungal opsonisation and subsequent phagocytosis by primary microglia was examined by immunofluorescence.

**Results:** When cultivated in CSF pathogenic *Aspergillus* species secreted proteolytic enzymes that are able to degrade a broad spectrum of complement factors of all three activation pathways. Besides soluble complement proteins, also complement receptor CR3 (CD11b/CD18) and MHC molecules on membranes of immune cells were affected. The consequences were striking: any new opsonisation of fungal hyphae was markedly reduced by CSF/Aspergillus supernatant, and complement factor C3 already bound to the surface was removed. In consequence microglial cells showed less phagocytic activity compared to conidia opsonised in CSF without fungal growth.

In order to prevent the secretion of fungal proteases, different supplements were tested. While sugars, phosphate and iron compounds showed no effect, various nitrogen sources were able to suppress the proteolytic activity. Amino acids as endogenous substances were of particular interest and turned out to be very effective; the number of the nitrogen atoms within the molecule seems to be crucial for the relevant concentration. Glutamine and arginine appear as the most promising candidates for a future therapeutic use as they are present in the brain and act in comparatively low concentrations.

**Conclusions:** The degradation of complement and surface-bound molecules of immune cells by secreted proteases turned out to be an important mechanism for immune evasion in cerebral aspergillosis. Inhibition or suppression of these proteases are promising approaches for supportive therapeutic approaches in the future.

**P909** Cumulative sums test for monitoring the incidence of invasive nosocomial aspergillosis


**Objective:** A close epidemiological surveillance of invasive aspergillosis (IA) is required in hospitals in charge of severely immunocompromised patients. In order to provide a statistically-based evaluation of IA incidence over time, we applied the Cumulative Sums (CUSUM) methodology, which was developed for quality control and has already been applied for the surveillance of hospital-acquired infections.

**Methods:** Cases of IA were prospectively recorded during a 5-years period and incidence rates were analyzed by CUSUM. Fungal monitoring of the environment (air and surfaces) was performed once every 3 weeks in haematology wards. The risk related to construction or renovation works was scored using a semi-quantitative scale with 4 levels of risk according to the type and location of works. Relationships between incidence rates, fungal contamination in haematology wards and risk-scores of works were tested by using time series methods.

**Results:** Between Jan 2002 and Dec 2006, 122 cases of probable or proven cases of IA were diagnosed; 41 cases had a previous history of aspergillosis and/or signs of aspergillosis at entry and the 81 other cases were called “nosocomial IA” (NIA) since hospital acquisition of infection could not be ruled out.

CUSUM analysis of NIA incidence showed no significant deviation from the expected monthly number of cases between Jan 2002 and Aug 2005. On Sept 2005, the CUSUM crossed the decision limit, i.e. identified a significant increase in risk of NIA as compared to the reference period (i.e. before Dec 2004). Up to April 2006 the Learning Curve CUSUM stayed over its limit, supporting an ongoing outbreak, then it showed a significant decrease on May 2006. Follow-up from May 2006 indicated no out-of-control situation, thus supporting a return to the baseline situation.

In the haematology wards, several significant links were found between the incidence of NIA and the degree of fungal contamination by *Aspergillus* or other fungi of several sites of each ward, especially the contamination of sites other than patient rooms. No significant relationship was found between NIA incidence and the construction or renovation works.

**Conclusions:** The CUSUM test is well suited for real-time monitoring of NIA, early identification of an ongoing uncontrolled process and assessment of the efficacy of control procedures. Fungal contamination, mainly in unprotected common sites of the wards might indicate a higher risk of NIA.

**P910** Usefulness of real-time PCR at mt LSU rRNA for the identification of *Pneumocystis jiroveci* colonisation

E. Campano*, R. Morilla, V. Friaiza, N. Respaldiza, M.A. Montes-Can, I. Martin-Garrido, F.J. Medrano, J.M. Varela, E. Calderón, C. de la Horra (Seville, ES)

**Background:** The accepted current diagnostic standard for *P. jiroveci* infection is the demonstration of the microorganism in sputum or bronchoalveolar lavage (BAL) samples using conventional stain or polymerase chain reaction (PCR). Besides oropharyngeal washes (OWs) are used to detect *P. jiroveci* colonisation by nested PCR in general population. Several studies have evaluated the use of Real time PCR to detect the MSG genes of *P. jiroveci*. The efficacy of this assay at mt LSU rRNA gene of *Pneumocystis* to detect the colonisation status was not being proved.

**Objectives:** To evaluate the usefulness of Real time PCR versus nested PCR using to detect *P. jiroveci* colonisation.

**Methods:** A total of 100 OWs specimens which included 23 samples colonised where *P. jiroveci* status was defined as samples identified by nested PCR at mt LSU rRNA gene at least twice in independent separate DNA extraction for each case. These samples were matched by colonisation status with 68 negative samples. Three controls colonised OW samples previously evaluated were also included. Nested PCR was performed using the consensus primers pAZ102-E and -H, and -X and -Y as internal primers. This protocol was adapted to a Real time PCR assay using only the PAZ102 -X and -Y primers.

**Results:** The data shows that mt LSU rRNA gene of *P. jiroveci* was detected by nested PCR in the three controls to 1:125 dilution of DNA target, and using real time PCR we are able to amplify to a 1:200 dilution. Among samples in study, twenty-three standard OW samples were positive for Pneumocystis using both methods, nested real time PCR. Likewise 64 samples were negative by both assays. Nine samples were positive using real time and in only one of the DNA extraction by nested. Finally 4 samples were only positives by real time PCR. The concordance of results was 100% respect to the positive samples and 94.9% respect to negative standard samples. Besides a 9% of samples were considered as indeterminate. The results show for the real time PCR respect to nested PCR a sensitivity of 100% and a specificity of 94.1% for the detection of *P. jiroveci* colonisation. The positive predictive value was 85.4% and the negative predictive value was 100%.

**Conclusions:** Real time PCR at the mt LSU rRNA gene may be a useful method because of its high sensitivity and negative predictive value this technique may be a valuable alternative to detect Pneumocystis specially when a large number of samples need to be analysed.
Hyper-pigmentation of blastospores in contrast to basidiospores of pathogenic cryptococci upon induction to ultraviolet radiation of near solar spectra

P. Yegneswaran Prakash*, P. Sugandhi Rao (Manipal, IN)

Objectives: To understand the effects of Ultra violet radiation exposure for controlled time intervals in the induction of hyper pigmentation in blastospores and basidiospores of pathogenic cryptococci.

Methods: A total of 15 strains isolated from clinical and environmental sources, representing the 5 serotypes and 3 varieties along with Cryptococcus neoforms 32045 from American Type Culture Collection and Cryptococcus albidus and Cryptococcus laurentii from environmental sources were used in the study. The blastospores were grown on sabourauds dextrose agar media and the basidiospores were using filamentation agar. Both the harvested blastospores and basidiospores were suspension in the defined minimal media with melanin precursors in the form of L-dopa. Blastospores and basidiospores were spread on to agarose sheet of 0.22 mm thickness and subsequently exposed to 315–400 nanometer wavelength mimicking solar rays with a control on exposure set for 10, 20, 40, 60 minute intervals. The copper sulfide silver staining was employed to record the hyper pigmentation after exposure to ultra violet rays subsequently data analyses performed by a Student t test using Statistical Package for Social Sciences version 11.0 software.

Results: The survivability of blastospores and basidiospores for shorter time period 10 to 20 minutes were in comparable limits. Longer exposure times 60 minutes were documented to be detrimental for both blastospores and basidiospores. At an optimal exposure of 40 minutes there was a statistically appreciable difference in the hyper pigmentation properties of blastospores P < 0.005. The Cryptococcus neoforms variety gatti blastospores was observed to be hyper pigmented compared to Cryptococcus neoforms variety grubii and Cryptococcus neoforms variety neoforms.

Conclusion: Radiation induced melanoprotective activity may play a role in the prolonged senescence and enhanced environmental survivability of the soil saprophyte like cryptococcus. The quest for melanin substrates might account for the central nervous system predilection of this basidiomycete in an infected host and hyper pigmentation the Cryptococcus neoforms variety gatti blastospores points to the increased virulence and and its ability to infect an apparently immunocompetent host.

Fungal metabolites can fuel an underlying HIV infection

C. Spath*, G. Rambach, M. Hagletier, I. Mohsenipour, R. Würzner, C. Lass-Flörli (Innsbruck, AT)

Objective: Invasive aspergillosis is a dangerous and highly lethal opportunistic infection in immunosuppressed HIV-infected patients. Nothing is known about a putative cross-effect between fungal and viral infection. A deeper insight into pathogenic mechanisms is urgently needed for an adaptation of antiviral therapy in the case of invasive aspergillosis. Therefore we studied whether fungal metabolites influence HIV replication and thus contribute to a progression to AIDS.

Methods: HeLa cells transfected with the HIV promoter, primary microglia, M1866 lymphocytes and THP-1 monocytes were pre-incubated with purified fungal metabolites followed by infection with HIV. Viral replication was analysed by quantification of promoter activity and by quantification of p24 in the culture supernatants. Toxicity and cell activation was controlled by measuring mitochondrial activity (MTS test).

Results: Subtoxic concentrations of the fungal metabolites gliotoxin and citrinin, known to be produced by pathogenic Aspergillus species markedly stimulated the cellular activity. Furthermore the two compounds both strongly enhanced HIV infection of HeLa cells as shown by the increased numbers of infected cells and of generated syncytia. The viral load in the culture supernatants was amplified after pre-incubation with gliotoxin or citrinin compared to mock-treated control cells. A similar effect of gliotoxin was visible for the infection and the production of progenitor virus with T-cells, monocytes and primary microglia. Other fungal metabolites like patulin, fumitremorgin or verruculogen were proven to be ineffective and did not influence viral infection and replication.

Conclusions: The fungal metabolites gliotoxin and citrinin boost and fuel the HIV infection of those cell types which represent viral targets in the patient, with a subsequent stimulation of progenitor virus production. Thus an opportunistic fungal infection of HIV-infected individuals should not only be treated with antivirymic drugs but also result in adaptation or initiation of antiviral therapy. Further knowledge in the pathogenic mechanisms of gliotoxin and citrinin might help to neutralise to metabolite-induced escalation of virus production and thereby inhibit disease progression in the patients.

Viral and fungal pathogenesis

Zinc: a modulator of influenza A virus induced programmed cell death

V. Srivastava*, M. Khanna (Lucknow, New Delhi, IN)

Objective: Programmed cell death (apoptosis) is a hallmark event observed upon infection with many viral pathogens, including Influenza A virus. Zinc is known as a potent inhibitor of programmed cell death. However, the effect of zinc on influenza A virus induced apoptotic death is not well established.

Methods: HeLa cells were infected with a cell adapted pathogenic strain of Influenza A (/Udorn/317/72H3N2) virus. Infected and mock-infected cells were treated with 0.1, 0.15 and 0.20 mM zinc at various time intervals. DNA fragmentation and Caspase-3 activity was examined at various time periods. The morphological changes and ultrastructural changes were studied staining with Haematoxylin-Eosin and Transmission Electron Microscopy (TEM) respectively. Annexin V assay was carried out for analysis of phosphatidylserine externalisation. Phagocytic index was determined by incubating infected cells with adherent mouse peritoneal macrophages.

Results: DNA fragmentation was observed in virus infected cells by 24 hours post infection. Caspase-3 activity was maximum at 4 hours post-infection after that it reached to plateau. It was observed that when the infected HeLa cells were incubated with adherent macrophages, efficient phagocytosis occurred and the release of virus into the culture medium was completely inhibited. Furthermore, TEM analysis detected phagosome-like structures within macrophages, which coexisted with the peak of phosphatidylserine externalisation i.e. 9–12 hours. Treatment of cells with 0.15 mM concentration of zinc inhibited DNA fragmentation till 8 hours of post infection and caspase 3 activity was decreased significantly up to 2 hr post infection.

Conclusion: These results suggest that the influenza A virus induces apoptosis in cell culture, thus apoptosis may represent general mechanism of cell death in infected host cells. Zinc has its inhibitory effect on caspase 3 and endonucleases both. Therefore, zinc modulated apoptosis in a time and dose dependent manner by inhibiting executioners caspase3 and endonucleases.

Analysis of microRNA expression in cytological cervical samples and correlation with HPV infection

V. Militello, E. Peta, M. Trevisan, S. Pagni, L. Barzon, G. Puliti* (Padua, IT)

Objectives: MicroRNAs play an important role in carcinogenesis. Recent studies have shown abnormal microRNA expression in cervical cancer cell lines carrying human papilloma virus (HPV) infection and suggested abnormal expression was related to HPV-16 E6 oncogene.

Methods: Cervical swabs from 40 females (age range: 17–56 years; mean age: 34.5 years) with negative Pap test were analysed for the presence of HPV DNA by PCR and HPV-positive samples were genotyped by sequencing of the HPV L1 gene. Moreover, the expression profile of 20 microRNAs, which were selected because reported to
be abnormally expressed in cervical carcinoma cell lines or because correlated with tumorigenesis and P53 activity, was investigated in all samples by real-time PCR using TaqMan® MicroRNA Assays (Applied Biosystems). The expression levels of each miRNA was calculated by the 2−ΔΔCt method against two different housekeeping snRNAs.

Results: Out of 40 samples, 10 were HPV-negative, 10 had low-risk HPV and 20 had high-risk HPVs, including 13 samples with HPV-16. Analysis of microRNA expression showed that let-7a/b/c, miR-21, miR-210 and miR-34a were abundantly expressed in all cervical samples, the microRNAs let-7a, let-7c, and miR-34b/c were frequently underexpressed, while miR-126, miR-143, and miR-368 were overexpressed in samples positive for HPV and for other high-risk HPV types as compared with HPV-negative samples or with low-risk HPVs.

Conclusion: The results of this study provide new information on HPV-related carcinogenesis and potential new diagnostic markers. The abnormally expressed microRNAs are involved in pathways that are altered in HPV-related carcinogenesis; in fact, underexpression of miR-34b/c might be the consequence of P53 downregulation by E6; let-7a and let-7c downregulation could contribute to the effect of E6 on cell growth induction; overexpression of miR-126 and miR-143 could cooperate with E6 to inhibit expression of the their target genes encoding PDZ domain-containing proteins involved in the regulation of cell polarity.

Objective: To compare the prevalence of EBV and CMV in tonsils among patients with recurrent tonsillitis and tonsillar hypertrophy (recurrent tonsillitis and tonsillar hypertrophy) was tested using the Fisher's exact test. A result was considered significant when \( P < 0.05 \).

Results: Sixty two specimens were found to be positive for the presence of EBV-DNA (32.98%). No significant difference was found concerning the presence of EBV-DNA between the two groups studied; 32.91% in patients with recurrent tonsillitis versus 26.47% in patients with tonsillar hypertrophy. On the other hand, CMV was not detected in any specimen.

Conclusion: The high prevalence of EBV among patients with recurrent tonsillitis and tonsillar hypertrophy verify that the tonsils are a potential reservoir for EBV infections.

Objective: Yeast SEC14 genes encode phosphatidylinositol transfer proteins that link phospholipid turnover with export of secretory vesicles from the Golgi complex by undefined mechanism(s). In both \( \text{Saccharomyces cerevisiae} \) and \( \text{Candida albicans} \), SEC14 protein is essential for growth, hindering delineation of its role in secretion. The aim here is to investigate the role of SEC14 in secretion of the fungal invasin, phospholipase B1 (Plb1), and in virulence of the model fungal pathogen, \( \text{C. neoformans} \).

Methods: The coding region of the putative SEC14 gene and two homologues, SFH1 and SFH5, were disrupted in \( \text{C. neoformans} \) creating the strains d-sec14, d-sfh1 and d-sfh5. Plb1 protein/activity was assessed by western blotting with an anti-Plb1 peptide antibody/radiometric enzyme assay. Cryptococcal virulence was investigated in a mouse model of cryptococcosis and in a macrophage killing assay.

Results: Similar to wild type (WT), all mutants grew at 37 degree Celsius and produced melanin. Only d-sec14 exhibited a cell wall defect and secreted less total protein and almost no Plb1 protein, indicative of a secretion block, and these phenotypes were also observed for a phospholipase C1 (PLC1) deletion strain [1]. As Plb1 is anchored to membranes and cell wall prior to secretion, Plb1 associated with these fractions was assessed by western blotting to determine the site of the secretion block in d-sec14. Results confirmed that, compared with WT, mature fully-glycosylated Plb1 (120 kDa) had reduced association with cell walls and, together with a smaller 40 kDa form, accumulated in the membrane fraction. As this fraction potentially contains plasma membrane (PM) and Golgi-associated Plb1, the origin of the secretion block could be either Golgi or PM. However, the presence of potentially degraded Plb1 suggests that the block occurs at the Golgi. Finally, d-sec14 was hypovirulent in mice and exhibited reduced survival in the presence of a macrophage cell line (J774.1). All d-sec14 phenotypes were restored by genetic reconstitution with either an intact SEC14 or a SFH1 gene which share 86% amino acid identity.

Conclusion: SEC14 regulates secretion of the fungal invasin, Plb1, maintains cell wall integrity, and is a novel virulence determinant in \( \text{C. neoformans} \), highlighting it as a potential antifungal drug target. Investigation into whether SEC14 and PLC1 co-ordinately regulate secretion of virulence determinants is underway.

Reference(s)
reconstituted in 0.1% acetic acid and stored in working aliquots ranging from 1 to 0.002 mg/ml.

Fungal strains & AFST. The filamentous fungi used in this study were *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus nidulans* and *Aspergillus terreus*, deriving from suspected cases of invasive mycotic infections. Presumptive species identification was done microscopically and their carbon assimilation profiles using the ID32C system (Biomérieux, Marcy d’Etoile, France). Routine antifungal susceptibility testing (AFST) for itraconazole, fluconazole, posaconazole, caspofungin and amphotericin B were performed by means of E-test (AB Biomérieux) according to the manufacturers’ instructions.

Antifungal defensin assay. Minimum Fungicidal Concentrations (MFC) of hbD1, hbD2 & hbD3 were determined by the microdilution assay as described elsewhere. Briefly, fungal spores were harvested from mature colonies grown on SDA agar at 30°C and resuspended in 10 mM sodium phosphate Buffer (PB) to obtain a final concentration of 10⁶ cells/ml. Aliquots of diluted cells were exposed to varying concentrations of defensins for 24 h at 30°C in sterile 96-well microtitre plates and growth inhibition was assessed by measuring culture absorbance at 595nm using a microplate reader. Cell suspensions in buffer alone and/or acetic acid served as negative controls.

Results: Inhibition of fungal growth was evident with all three defensins tested and was accompanied by fungicidal effects as demonstrated by re-inoculation of the fungi to SDA plates. Among those tested, hbD3 appears to have the strongest potency as compared to those of hbD1 & hbD2.

Conclusions: In recent years, intensive work on defensins has expanded our knowledge on innate defence mechanisms and may provide us novel therapeutic options for combating serious and emerging infections as invasive mycoses. The present study, provides a first indication of specific antifungal activity of those endogenous peptides against clinically important filamentous fungi and highlights the need for further analysis of their mode of action.

Formation of stomatopathies in correlation with presence of *Candida* and *Lactobacillus* strains

M. Brzychczy-Wloch *, M. Lacki, S. Majewski, P. Heczko (Cracow, PL)

Objectives:
1. To identify species composition of *Candida* and *Lactobacillus* strains isolated from patients with a total lack of dentition using a plate dental prosthesis.
2. To indicate the reciprocal relationship between the presence of *Candida* and *Lactobacillus* and the formation of stomatopathies.

Methods: The study was performed on a group of 100 persons living in the South-Eastern region of Poland. Inclusion criteria were total lack of dentition and using a plate dental prosthesis during a min. 6 months period. Group I (n=53) were patients with inflammation of the mucous membrane of the prosthetic base, Group II (n=47) were patients without stomatopathy. We collected palate swabs. The materials were cultured on Sabouraud and MRS agar. The *Candida* and *Lactobacillus* strains were identified using phenotypic methods (API20C AUX, API50CHL). The identification of Lactobacillus was confirmed by PCR.

Results:
1. Higher percentage of positive cultures of yeast-like fungi in patients with stomatopathies (67%) was statistically significant (p < 0.0001) in relation to patients without stomatopathies (28%).
2. Between isolated yeast-like fungi strains, *Candida albicans* species dominated in patients with stomatopathy (87%) or without stomatopathy (67%).
3. The differences in composition and number of *Lactobacillus* species in both examined groups were found. From patients with stomatopathies ten different species of *Lactobacillus* genus were isolated: *L. fermentum* 32%, *L. salicarius* 21%, *L. rhamnosus* 12%, *L. brevis* 10%, *L. acidophilus* 7%, *L. para paracasei* 7%, *L. fermentum* 2%, *L. delb. lactis* 2%, *L. lact. lact. 2% whereas from patients without stomatopathies five species were isolated: *L. fermentum* 39%, *L. plantarum* 34%, *L. para paracasei* 17%, *L. rhamnosus* 5%, *L. salicarius* term. 5%.
4. A significant correlation between the presence of *L. plantarum* strains and the lack of stomatopathy was confirmed (p = 0.0012).

Conclusions: Stomatopathies are patological lesions which give characteristic symptoms and these are triggered by using a plate dental prosthesis and appearance of yeast-like fungi. Our results confirm significant influence of mycotic infection on induction of the stomatopathies. Moreover, a statistically significant correlation was demonstrated between the presence of *L. plantarum* and lack of stomatopathies. The following step which should be undertaken is to confirm these results in a clinical trial with administering the probiotic strains representing these species of bacteria.

Sero-prevalence of Rift Valley fever in south-western Saudi Arabia and study of risk factors

T. Alszaqui *, A. Mahfouz, A. Meeki (Abha, SA)

Seroepidemiological studies are valuable tools to identify the state of immunity among the general population to Rift Valley Fever (RVF) in the affected zones. The objectives of the present research were to study the seroepidemiology of RVF infection in Jizan, Aseer and Al Qunfudah regions (Southwest OF Saudi Arabia), potential risk factors leading to the infection and to elucidate the predisposing factors of developing severe RVF disease requiring hospitalisation. Through a series of field trips, (during the period September 2007–June 2008) to the centres selected for the present study (Jizan, Abu-Areesh, Al-Arida, Samtah, Beesh, Al-Birk, Al Gahma, Muhayeel, Al-Majardah and Al-Qunfudah) a random sample of individuals attending the outpatients’ clinics for any reasons were included. All abattoir workers in those regions were also enrolled. Through questionnaire interviews, data were collected.
Blood samples were taken and tested for RVF-specific IgG and IgM utilising commercially available enzyme-linked immunosorbent assays (ELISAs). Out of 2,322 persons included in the study, only 137 were positive for RVF-specific IgG thus giving an overall prevalence of 6.0%. On the other hand, none of the study samples were found to be sero-positive to RVF-specific IgM. The highest prevalence of sero-positive RVF IgG was observed in Al Birk of Aseer region (13.3%) followed by Al Arda of Jizan Region (11.8%), where the first animal deaths were reported during 2000–2001 outbreaks. The study revealed zero prevalence of specific IgM and IgG among pre-school children born after the 2000–2001 outbreak. Using multivariate binary logistic regression analysis to identify potential risk factors associated with seropositive RVF IgG, the following significant risk factors were identified: lacking electricity, having animals in the house, history of slaughtering animals, contact with or transporting aborted animals.

The study included a retrospective cohort of 61 cases with severe RVF hospitalised in Aseer Central Hospital during the outbreak of 2000–2001. Results revealed that prior hepatic (HBV, HCV) and renal involvement on admission were strong predictors of poor outcome. In conclusion, the study documented a seroprevalence of 6% of RVF-infection hospitalised in Aseer Central Hospital during the outbreak of 2000–2001. Results revealed that prior hepatic (HBV, HCV) and renal involvement on admission were strong predictors of poor outcome. In conclusion, the study documented a seroprevalence of 6% of RVF-infection hospitalised in Aseer Central Hospital during the outbreak of 2000–2001. Results revealed that prior hepatic (HBV, HCV) and renal involvement on admission were strong predictors of poor outcome.
**P925** Molecular characteristic of *Staphylococcus aureus* associated with bacteraemia in Poland during 2005  

**Objectives:** The objective of the study was characteristics of bacteraemia-associated methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA).

**Methods:** Between January and May 2005 a total of 915 *S. aureus* isolates were recovered from 39 hospitals located in different areas of Poland. Clinical strains were isolated from the following specimens: wound, blood, respiratory tract, bone and joint, urine, and cerebrospinal fluid. Further phenotypic and molecular investigations were performed on blood culture isolates only (n=146). All isolates were characterised by an RM PCR based test and analyzed by spa typing. MRSA isolates were confirmed by PCR for mecA. Selected MRSA and MSSA isolates (nontypeable by both spa and RM tests) were typed by the MLST method. The presence of luk-PV genes was detected by PCR.

**Results:** Among the 108 MSSA over 50 different spa types were identified. The most common spa types were t008 (10.2%), t127 (8.3%), t091 (5.5%), t008 (3.7%), and t012 (2.7%). The all spa types were clustered into 10 different clonal complexes (CCs), four singletons, and 10 spa types that were excluded from the analysis, because the spa locus was less than five spa repeats in length. Up to 30% of the MSSA had a background observed in four major international MRSA lineages: CC5, CC8, CC30, and CC45. Several MSSA lineages were observed that were not associated with MRSA, such as: CC1, CC7, CC12, CC15, CC25, and CC51. In contrast, simultaneously circulating MRSA strains (n=38) belonged to 10 different spa types and were classified to three CCs: CC8, CC5, and CC45. One MRSA isolate with spa type t437 was nontypeable by RM testing. It was classified to CC59 (ST338-Vclone) according to MLST. PVL coding genes were detected among MSSA of CC15 and MRSA of CC59, which is a common CA-MRSA lineage worldwide.

**Conclusion:** MRSA strains belonging to CC8 were predominant among isolates associated with bacteraemia during the study period. In contrast, among MSSA isolates, strains belonging to CC15 (ST15) were predominant. MSSA were more diverse genotypically, including representatives of four major MRSA clonal complexes (CC5, CC8, CC30, and CC45). PVL, detected in only two isolates, did not seem to be an important factor in the pathogenesis of staphylococcal bacteraemia.

**P926** Molecular characteristics of PVL–positive community acquired methicillin-resistant *Staphylococcus aureus* strains isolated in the Czech Republic  
M. Fridrichová* (Prague, CZ)

**Objectives:** Community-acquired MRSA (CA-MRSA) strains producing Panton-Valentine leucocidin (PVL) have a propensity to cause skin and soft tissue infections of considerable severity. The aim of the study was to describe the presence and the clonality of CA-MRSA PVL-positive isolates collected in our laboratory in past three years.

**Methods:** Forty nine national microbiology laboratories participating in the European Antimicrobial Resistance Surveillance System (EARSS) submitted MRSA isolates (n=879) collected from individual patients in 2006 to 2008 to the National Institute of Public Health. The strains were identified by conventional phenotyping methods, the resistance to oxacillin was confirmed using chromogenic screening plate (Oxoid) and by PCR detection of mecA gene. The presence of PVL encoding genes was performed by PCR. 24 PVL positive MRSA isolates were further characterised by SCCmec typing, *S. aureus* protein A (spa) sequencing and MLST. Antimicrobial susceptibility testing was determined by microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI).

**Results:** Altogether 24 (2.7%) of 879 MRSA strains had the genes encoding PVL. The strains were collected mainly from skin lesions (19 isolates), 4 strains were recovered from blood, and one strain was isolated from nose. The type IV of SCCmec cassette was the most predominant among isolates (n=16), remaining strains possessed type V (n=4), type II (n=2) and type VI (n=1); one strain was untypeable. Spa typing and MLST typing was highly congruent. Molecular typing revealed the presence of 4 clonal groups, 5 strains were singletons. The most prevalent ST’s and spa types were as follows: ST 8, spa type t008 (n=8); ST 5, spa type t002 (n=3); ST 80, spa type t044 (n=3) and ST 88, spa types t186 and t690 (n=2).

**Conclusion:** Our survey revealed the presence of CA-MRSA PVL positive strains in the Czech Republic. The most prevalent type identified in set of Czech strains showed sequence and spa type usually associated with USA300 clone. The other clonal lineages previously reported in CA-MRSA strains have been also observed. This work was supported by a research grant from the Internal Grant Agency, Ministry of Health (CZ), IGA 9642–4.

**P927** Emergence of “animal” MRSA ST398 as coloniser and as infectious agent in humans  
C. Cuny, B. Strommenger, W. Witte* (Wernigerode, DE)

**Objective:** To assess the frequency of colonisation by of humans professionally exposed to MRSA ST398 and of their family members.

**Methods:** Investigation of nasal swabs from farers and veterinarians as well as of their family members, typing by means of spa-typing and grouping of SCCmec elements.

**Results:** In 32 farms in all Germany: 73 (54.4%) humans from 134 humans exposed to MRSA colonised pigs and 7 (5.4%) of their nonexposed family members carried MRAST398. In 24 families with veterinarians exposed to MRSA positive pigs 28 (57%) from 49 were positive for MRSA ST398 and this was also found in 3 (4.7%) of their nonexposed family members. The frequency of MRSA ST398 among all MRSA from various kinds of infections in humans sent to The German Reference Center for Staphylococci for typing was 0.3% with two cases of septicaemia included besides infections of skin and soft tissue which needed surgical intervention.

**Conclusion:** As shown in a number of studies in other European countries MRSA ST398 is a frequent nasal coloniser of humans exposed to colonised animals. It is obviously also able to spread to contact persons, in one case emerging during this study the patient died after wound infection and septicaemia. In general MRSA ST398 is still rare among MRSA from infections in humans.

**P928** Detection of cfr-carrying *Staphylococcus aureus* spp. isolates in Blood Cultures from a Spanish site during a phase 3 clinical trial of topical administration of omiganan 1% gel  
R. Mendes*, M. Sanchez, L. Deshpande, M. de la Torres, R. Jones (North Liberty, US; Madrid, ES)

**Objectives:** Central venous catheter related infections (CRI) are often caused by skin flora. Prevention of catheter colonisation by skin flora and local catheter site care are crucial steps for controlling CRI. Omiganan, a topical cationic peptide, is being studied for this indication in a large, multicentre phase 3 trial. We report detection of cfr-carrying staphylococci from a patient during the trial. Experiments were conducted to evaluate potential in vivo cfr transfer.

**Methods:** *Staphylococcus* spp. isolates were tested for susceptibility by CLSI broth microdilution methods. Identification was performed by Vitek 2. Isolates displaying linezolid MIC at ≥4mg/L were screened for mutations at the 23S rRNA, L4 and L22 genes, followed by sequencing and for cfr. Primers targeting the sequences previously detected in the cfr-carrying plasmid pCF53 were used to PCR map the cfr surrounding region. Gene location was accessed by Southern blot and hybridisation.

**Results:** A 77(yo) male patient was hospitalised in the ICU due to respiratory failure. Patient received broad-spectrum antimicrobial therapy, including linezolid. One *S. epidermidis* and one *S. aureus* were recovered on the hospital day 23 and 26 from catheter and peripheral drawn blood specimens, respectively. Isolates were susceptible...
to omiganan (MIC, 8 and 32 mg/L) and resistant to mucopein, neomycin, cipriofoxacin, erythromycin, gentamicin and oxacillin. Both strains were resistant to chloramphenicol, clindamycin, linezolid, tetracyclum and quinupristin/dalfopristin, consistent with cfr. Ribosomal target mutations were not detected. Plasmid analysis identified two bands in each isolate of c.a. 160- and 250-, and 145- and 190-kb. Hybridisation signals with a cfr-probe were noted from the 250-kb and 190-kb bands. PCR mapping identified delta tapl downstream of the cfr in the S. epidermidis. Both isolates did not possess the istAS and istBS structures associated with the cfr mobility.

Conclusion: cfr were embedded in different size plasmids and both genes showed distinct surrounding sequences, suggesting diverse acquisition events. This report highlights the ability of staphylococci to acquire multidrug resistance and the potential for cfr dissemination, representing a serious threat against current Gram-positive agents. The MIC values for these isolates were well below the concentration of omiganan 1% gel (10,000 mg/L) used in this trial.

Methods:

Objective: M. Martsoukou, E. Papafragas, E. Petinaki (Larissa, Athens, GR)

Results:

S. epidermidis was resistant to oxacillin, cefotxin, tobramycin, gentamicin, ciprofloxacin, ofloxacin, fusidic acid, erythromycin and clindamycin, intermediate resistant to linezolid and susceptible to vancomycin, teicoplanin, tetracycline, daptomycin, teicoplanin, fusidic acid and tigecycline. The MICs for vancomycin, teicoplanin were 12, 32, 1 and 1 mg/L, respectively. The isolate did not carry the G2576T mutation, neither the cfr gene and any mutations on ribosomal protein L4 and L22 genes. Analysis of 23S rRNA DNA sequences showed that, our isolate had two out six copies of 23S rRNA gene (the rrlA and rrlC) that carried the C2534T mutation; none of them was found to contain one or more mutated alleles.

Conclusions: A new mutation, the C2534T, of the domain V of 23S rRNA, was identified in a clinical linezolid-resistant S. epidermidis. This finding emphasizes that Gram-positive cocci under the pressure of linezolid had the possibility to develop various ribosomal mutations, other than the G2576T.

In vitro resistance of Gram-positive cocci

S. epidermidis isolates

F. Campanile*, D. Bongiorno, S. Barbone, G. Mongelli, M. Baldi, R. Provenzani, S. Stefani (Catania, Palermo, IT)

Objectives: The mechanism of cfr-mediated resistance to linezolid involves the methylation of A2503 in the 23S rRNA of the large ribosomal subunit. This methylation affects the binding of at least three antimicrobial classes (phenicols, lincosamides, and streptogramin A), leading to a multi-drug resistant phenotype. The aim of our study was to investigate the rapid spread of S. epidermidis strains sharing high level resistance to linezolid.

Methods: The activity of linezolid and other comparator agents was evaluated against 18 clinical S. epidermidis isolates recovered during 2007–2008, from the ICU of the Hospital Villa Sofia in Palermo, by broth dilution (MICs) and E-test methods. Mutations in the domain V of the 23S rRNA or cfr-mediated linezolid resistance were confirmed by PCR and sequencing assays. Macrolide restriction analysis was performed by PFGE.

Results: The 18 clinical S. epidermidis, isolated from 14 patients, were methicillin-resistant, MDR and showed linezolid MIC values ranging from 16 mg/L to >256 mg/L, not related to the G2576T mutation of 23S rRNA. They all belonged to similar PFGE subtypes (A1-A3) and only 8 strains showed the cfr gene. The presence of this methylnase conferred full resistance to linezolid (MIC >256 mg/L) and related drugs. The cfr gene was localised on the pSCFS-like elements, down-stream of the IS21–558 insertion sequence that is involved in its mobilisation.

Conclusion: Even if cfr-mediated linezolid resistance among clinical isolates is rare, we demonstrate the cfr gene acquisition by 8 S. epidermidis strains of human origin and their rapid spread among patients of the same ICU, independently from the linezolid use. Non-mutation resistance to oxazolidinones, due to the cfr gene, has recently been reported in veterinary isolates of staphylococci, first described in S. sciuri and immediately detected in many other Staphylococcus spp. of animal origin, carried by pSCFS-like elements. The recent acquisition of the cfr gene by S. aureus and S. epidermidis human isolates has been described in only three cases, localised either on plasmids or a chromosome.

A C2534T mutation in the 23S rRNA gene responsible for linezolid resistance in a clinical Staphylococcus epidermidis isolate

A. Lisakopoulou*, C. Neocleous, M. Kanellopoulou, N. Skarmoutsou, M. Martsoukou, E. Papafragas, E. Petinaki (Larissa, Athens, GR)

Objective: In the present study the mechanism of resistance of a clinical S. epidermidis isolate, expressing resistance to linezolid (MIC: 12 mg/L) was investigated.

Methods: In September 2008, a S. epidermidis exhibiting resistance to linezolid was isolated from blood cultures of a patient hospitalised in the Intensive Care Unit at “Sismanoglion” General Hospital of Athens, after ten days exposure to linezolid. The identification of the isolate to species level was performed by conventional and molecular methods. Susceptibility testing for various antimicrobial agents was performed by disc diffusion using CLSI criteria, while, the determination of MICs to oxacillin, vancomycin, teicoplanin, and linezolid, was assessed by CLSI micro-dilution method and by E-test. After extraction of bacterial DNA, the isolate was firstly tested for the presence of the most common mechanisms: for the presence of G2576T mutation by PCR followed by NheI digestion, and for the presence of the cfr gene by PCR. In addition, the detection of some mutations in the region V of 23S rRNA and on ribosomal protein L4 and L22 genes was performed by PCR followed by sequencing analysis.

Results: According to disc diffusion test results, S. epidermidis was resistant to oxacillin, cefotxin, tobramycin, gentamicin, ciprofloxacin, ofloxacin, fusidic acid, erythromycin and clindamycin, intermediate resistant to linezolid and susceptible to vancomycin, teicoplanin, tetracycline, daptomycin, teicoplanin and tigecycline. The MICs to vancomycin and teicoplanin were 12, 32, 1 and 1 mg/L respectively. The isolate did not carry the G2576T mutation, neither the cfr gene and any mutations on ribosomal protein L4 and L22 genes. Analysis of 23S rRNA DNA sequences showed that, our isolate had two out six copies of 23S rRNA gene (the rrlA and rrlC) that carried the C2534T mutation; none of them was found to contain one or more mutated alleles.

Conclusions: A new mutation, the C2534T, of the domain V of 23S rRNA, was identified in a clinical linezolid-resistant S. epidermidis. This finding emphasizes that Gram-positive cocci under the pressure of linezolid had the possibility to develop various ribosomal mutations, other than the G2576T.

Continuous increase of antibiotic resistance among non-vaccine type Streptococcus pneumoniae in the era of 7-valent conjugate pneumococcal vaccine

S. Nunes*, R. Sá-Leão, A.S. Simões, N. Frazão, A. Tavares, H. de Lancastre (Oeiras, PT)

Objectives: We have been monitoring changes in the Streptococcus pneumoniae population inhabiting the nasopharynx (NP) of young children (up to 6 years) following introduction of the seven-valent pneumococcal conjugate vaccine (PCV7) in 2001. In 2006 and 2007 approximately 60% of the target population had received PCV7. Here we describe the most recent data obtained in Oeiras, Portugal in 2007.

Methods: Data on antimicrobial consumption and NP samples were obtained and pneumococci were isolated by routine methods. Antibiotic susceptibility testing and serotyping were performed.

Results: Of 538 children, 19% had taken antibiotics in the month preceding sampling and 334 (62%) were colonised with pneumococci. Eighty-six percent of the isolates were non-vaccine type (NVT) a value comparable to what was observed in 2006. However, the proportion of NVT resistant to at least one antibiotic increased significantly from 27% in 2006 to 37% in 2007. Furthermore, this increasing trend has been continuously observed since introduction of PCV7 when this rate among NVT was 16%. In particular, intermediate resistance to penicillin (0.12≤MIC≤1 µg/mL) increased from 6% in 2001 to 18% in 2006 and 23% in 2007. Resistance to penicillin among NVT was found in 1.4% of the isolates only. Resistance to macrolides (mainly MLSB phenotype) increased from 9% in 2001 to 22% in 2006 and 28% in 2007. Multidrug resistance in 2007 reached 26% of the NVT. In 2007 the most frequently NVT serotypes associated with resistance were 19A, 6C, 15A and 11A.

Conclusions: The relative proportion of NVT among the total pneumococcal population did not change from 2006 to 2007. However, rates of resistance to antibiotics continue to increase among NVT and apparently a plateau has not yet been reached. The data suggests that antibiotic pressure in this population is very high. Continuous surveillance is needed as well intervention strategies aimed to decrease antibiotic consumption.
**P932** Adult invasive pneumococcal disease in North-Rhine Westphalia, Germany, 2003–2006: serotype distribution before recommendation of general pneumococcal conjugate vaccination for children <2 years of age

M. Imiöhl*, R.R. Reinert, M. van der Linden (Aachen, DE; Paris, FR)

Objectives: This study was performed to analyse the current epidemiology of invasive *S. pneumoniae* isolates in adults before the general recommendation for vaccination of German children <2 years with the pneumococcal conjugate vaccine was issued at the end of July 2006.

Methods: A population and laboratory based surveillance study of adult (16 years and above) invasive pneumococcal disease was conducted in North-Rhine Westphalia, Germany's most populous federal state, with approximately 18 million inhabitants. Species confirmation was done by optochin testing, bile solubility and serotyping.

Results: Invasive isolates were obtained from 519 adult patients from 2003 to 2006. The leading serotypes were serotypes 14 (14.3%), 7F (9.4%), 3 (9.2%), 4 (8.7%) and 1 (8.1%). Serotype coverage for the 7-valent conjugate vaccine was 43.9%. For the 10-valent and 13-valent vaccines in development the coverages were 61.8% and 76.7%, respectively. The 23-valent polysaccharide vaccine had a coverage of 91.7%. Between G resistance was observed in 5% of meningitis cases. In the non-meningitis group only intermediate resistant strains were detected (0.4%). Cefotaxime intermediate resistance occurred in meningitis (1.7%) and non meningitis cases (0.4%). Non-susceptibility rates (intermediate resistance and resistance) were 16.2% for macrolides, 10.9% for trimethoprim-sulfamethoxazole (SXT), 5.0% for tetracycline, 3.9% for clindamycin and 0.4% for levofloxacin. All isolates were susceptible to amoxicillin (non meningitis) and telithromycin.

Conclusions: The present study describes the current status of IPD in adults in North-Rhine Westphalia, Germany, and may serve as a basis for the interpretation of potential changes concerning effects of pneumococcal childhood vaccination programmes on IPD in adults, especially on serotype distribution, resulting coverages of current vaccines and vaccines in development as well as their potential effects on antibiotic resistance of *S. pneumoniae*.

**P933** Serotypes evolution in *Streptococcus pneumoniae* strains isolated from respiratory tract infections in adults in France from 2001/02 to 2006/07

H.B. Drugeon*, E. Leput (Nantes, Beaucez, FR)

Objectives: To describe changes in serotypes of *Streptococcus pneumoniae* (SP) strains isolated in respiratory tract infections (RTIs) in adults in France between 2001/02 and 2006/07

Methods: Since 2001/02, a French survey evaluates each year (from October to March) the susceptibility of SP isolated from RTIs in adults to usually prescribed antibiotics. Serotypes were determined by Quellung test in 657 strains isolated in 31 laboratories in 2001/02 and by PCR in 1002 strains from 42 laboratories in 2006/07. Antibiotics MICs were determined by microdilution.

Results: In 2001/02, 46 serotypes or groups of serotypes were identified whereas in 2006/07, 29 serotypes or groups of serotypes were identified and 11% of strains were not determined even possessing the capsular gene. In 2001/02, 8 serotypes had frequencies above 5% (3, 6A, 6B, 9V, 14, 19A, 19F, 23F) vs. 6 serotypes (3, 6A, 14, 19A, 19F, 23F) in 2006/07. Between the two periods, the frequency of 23F has decreased from 17.05 to 6.1% and the frequencies of 2 serotypes (11A and 35B) have increased and were higher than 3% in 2006/07. Between 2001/02 and 2006/07, intermediate resistance to penicillin was observed in 14 and 14 serotypes and high resistance in 9 and 15 serotypes respectively. Regarding amoxicillin, intermediate resistance was observed 9 and 13 serotypes and high resistance in 6 and 9 serotypes respectively. Vis-à-vis the macrolides, a mechanism of resistance was found in 21 and 19 serotypes respectively.

Conclusion: Frequencies of some epidemic and vaccine serotypes (6B, 9V, 23F) significantly decreased. The resistance to [lactams (low and high level) spreads and was observed in a greater number of serotypes in 2006/07. The mechanisms of resistance to macrolides were largely present amongst numerous serotypes but their number decreased slightly.

**P934** The serine threonine kinase StkP of *Streptococcus pneumoniae* contributes independently from pbp genes to penicillin susceptibility

R. Dias*, D. Félix, M. Caminha, M. Trombe (Lisbon, PT; Toulouse, FR)

Objectives: The PBPs are the major targets for penicillin in *Streptococcus pneumoniae*. The serine/threonine kinase StkP of *S. pneumoniae* was described to be one target of the phosphoglucomutase GlnM involved in peptidoglycan biosynthesis, being implicated in [lactams susceptibility. In order to further elucidate the association of StkP in *S. pneumoniae* in susceptibility to [lactams, mutational analysis was undertaken as well as the analysis of genetic diversity of StkP and PBPs of a sample of clinical strains.

Methods: A set of isogenic mutants was constructed in RX derivatives with different combinations of PBPs and StkP alleles. The conservation of StkP and PBPs was assessed for 50 strains randomly selected between 1994 and 2005 in various areas of Portugal. Half of the isolates were non-susceptible to penicillin (minimal inhibitory concentration, MIC > 0.1 mg/L). These isolates were compared to seven reference strains. The MIC of penicillin G was determined by agar dilution method according to Clinical and Laboratory Standards Institute. Genetic polymorphism of penA, pbpX and pbp1A genes was investigated by restriction fragment length polymorphism analysis, and by nucleotide sequencing. The average evolutionary divergence of StkP within penicillin susceptibility was estimated by the Poisson Correction method and the Maximum Composite Likelihood method for the amino acid and nucleotide substitutions, respectively. To further understanding the role of each founded StkP amino acid mutations, a 3D-model of the kinase domain of the StkP protein was used.

Results: Deletion replacement mutation in stkP conferred hypersensitivity to penicillin G and was epistatic on mutations in PBP2X, PBPB2 and PBPA1 from the resistant 9V clinical isolate URA1258. Genetic analysis of 55 clinical isolates allowed identifying 11 StkP alleles with regard to the reference R6 allele. Four alleles (StkP alleles: 3, 7, 10 and 11) were only found in sensitive strains. Nevertheless, these strains showed PBP profile characteristic of sensitive strains, suggesting that MICs were determined by their PBPs rather than mutations in StkP.

Conclusions: These findings reveals that StkP is involved in the bacterial response to penicillin and suggests that StkP activity allows bacteria to bypass cell wall injury due to penicillin up to a critical concentration independently of PBPs for a given strain. It is also suggested strong functional conservation of StkP among clinical isolates.

**P935** Characterisation of genetic elements carrying mer other than mer(A) in *Streptococcus pyogenes*

M. Del Grosso*, G. Barbabella, R. Camilli, J. Blackman Northwood, D.J. Farrell, A. Pantosti (Rome, It; Cambridgeshire, UK; Toronto, CA)

Objectives: In *Streptococcus pyogenes*, erythromycin (ERY) resistance due to efflux is mainly associated with the mer(A) subclass of the mer gene, that is carried by a conjugative transposon/prophage. The aim of this study was to characterise the genetic elements carrying mer subclasses other than mer(A), in selected *S. pyogenes* isolates.

Methods: From a global collection of clinical isolates of *S. pyogenes*, 10 isolates carrying mer other than mer(A) were selected: 4 were resistant to ERY and tetracycline (TET), 5 to ERY and chloramphenicol (CHL), and 1 to ERY, TET and CHL. PCR assays were performed to detect the resistance genes and to define the possible linkage among them. To verify the presence of composite genetic elements previously described in other bacterial species, PCR mapping was performed.

Results: The 4 ERY-TET resistant isolates carried mer(E), rarely reported in *S. pyogenes*, and tet(M). The 5 ERY-CHL resistant isolates carried mer(I), a mer subclass recently described, and catQ. The ERY-TET-CHL resistant isolate carried a novel subclass of mer, tet(M), and catQ.
Physical linkage among the resistance genes was observed. Analysis of the 10 isolates by PCR mapping, indicated the presence of genetic elements, or part of them, harbouring the resistance genes. In the 4 mefE-tem(M)-positive isolates, mefE was carried by a typical mega element that was found inserted in orf6 of Tn916, showing the genetic organisation of Tn2009 described in Streptococcus pneumoniae. In all the mef(I)/catQ-positive isolates the linkage between mef(I) and catQ was observed. PCR mapping indicated the same structure of the IQ element described in the 5216IQ complex of S. pneumoniae. The novel mef(TN)/catQ-positive isolate showed the same genetic structure of the 5216IQ complex with the exception of a 4 kb-deletion.

Conclusion: This study showed the presence in S. pyogenes of two composite elements, Tn2009 and the 5216IQ complex, that have not been reported before. These findings enlarge the number of genetic elements and resistance modules shared between S. pyogenes and S. pneumoniae.

**Tn1806, the erm(TR)-carrying genetic element of Streptococcus pneumoniae**


Objectives: Tn1806 is the first erm(TR)-carrying genetic element reported in a clinical isolate of Streptococcus pneumoniae (strain AP200) and to date has been only partially characterised. In the context of a whole genome sequencing project of the pneumococcal AP200 strain, the complete nucleotide sequence of Tn1806 has been obtained. Aim of this study is to give a fully characterisation of Tn1806.

Methods: The Tn1806 sequence was obtained by high-density pyrosequencing and comparative analysis was carried out using the BLAST algorithm.

Results: Tn1806 is 52,457 kb in size and comprises 49 ORFs. Tn1806 is inserted into the hsdM chromosomal gene coding for a restriction modification methyltransferase. At the insertion sites a duplication of the target sequence for integration of the element has been observed. In the region flanking erm(TR), Tn1806 carries other antibiotic resistance genes such as the tet(M) efflux pump and a spectinomycin fosfotransferase. Comparative nucleotide analysis confirmed similarity of Tn1806 with ICE10750 RD-2, the composite element able to incorporate resistance genes, thus contributing to the diffusion of multiple drug resistance.

Conclusion: Tn1806 is the first erm(TR)-carrying genetic element of S. pneumoniae. Comparative nucleotide analysis confirmed similarity of Tn1806 with ICE10750 RD-2 due to the presence of additional regions such as 3 ORFs found also in RD1, a chimeric element of S. pyogenes MGA6180. Comparative analysis detected an element similar to Tn1806 in Finegoldia magna, an anaerobic Gram-positive coccus. The F. magna element shares high nucleotide similarity over a region of about 23 kb but lacks the region carrying erm(TR) and the other antibiotic resistance genes, which is replaced by a different sequence of similar size. A large region similar to Tn1806, has been found also in the incomplete genome of Ureaplasma urealyticum ATCC 33175. Tn1806, ICE10750 RD-2 and the F. magna element share a similar backbone structure, with some insertion/deletion or replacement of modules that confer element-specific features. Most of the unique modules differ Tn1806/ICE10750 RD-2 from the F. magna element carried genes related to drug resistance such as those conferring resistance to erythromycin, tetracyclin and spectinomycin in Tn1806 and ICE10750 RD-2 or a series of ABC multidrug resistance efflux pumps in F. magna. The common backbone structure shared by Tn1806, ICE10750 RD-2 and the F. magna element could represent a broad-host-range element able to incorporate resistance genes, thus contributing to the diffusion of multiple drug resistance.
Epidemiological analysis of pneumococcal carriage in Hungarian children from a day-care centre

O. Dobay*, A. Töthpály, S. Kardos, E. Hajdú, E. Nagy, S. Amves, K. Nagy (Budapest, Szeged, HU; Edinburgh, UK)

Objectives: The carriage of Streptococcus pneumoniae (pneumococci) plays a major role in the transmission of bacteria to sensitive individuals. The carriage rate can reach 50–100% in small children, especially attending day-care centres. Prevenar was proven also to have an effect for children <2 y in Oct 2008, it was important to examine the carriage of pneumococcal strains before the vaccination programme was implemented.

Methods: Thirty-four pneumococcal isolates were collected from the nasal passages of children attending a day-care centre in Szeged, Hungary. The species identity of all strains was confirmed by the presence of the lytA gene. Their antibiotic sensitivity was determined by E-test, applying the EUCAST breakpoints. Serotyping was done by the combination of the conventional method (with antisera) and a PCR-based method. The genetic relatedness of the strains was examined by PFGE.

Results: We could serotype 25 out of the 34 strains (≈73.5%). The detected serotypes were (n): 14 (6), 6A (6), 3 (3), 13 (2), 18C (2), 9L, N (2), 9V (1), 19A (1), 19F (1), 15A (1). None of the strains were resistant (R) to penicillin (pen), the highest MICs (0.5–0.75 mg/L) were detected in sero 14 isolates. Serotypes 6A and 19 also fell in the pen I category. All isolates were sensitive (S) to levofloxacin. Seven strains showed high-level R to erythromycin (ery), these were of different serotypes, and 4 carried the erm(B) gene. Five strains were R to ery (6–24 mg/L), but S to clindamycin (M type), all these were of sero 14, identical by PFGE and had the mef gene. One single sero 14 strain was S to everything, and shared PFGE identity with one sero 13 strains. The six sero 6A strains formed 2 PFGE clones, mirroring the different pen and ery MICs.

Conclusions: It is surprising to observe the absence of the usually frequent serotype 23F among the carried strains, and the presence of the rather rare serotype 13. Based on our data, and taking certain cross-protections into account, Prevenar (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) would cover 18 out of the 25 isolates (72.0%), or 52.9%, if we relate it to all 34 strains. The presence of a mef- serotype 14 clone (very probably the England 14–9 PMEN clone) was observed. An interesting serotype switch was detected between sero 14 and 13. This study involves only a small number of isolates; however, this is the first study on pneumococcal carriage in Hungary.

Methods: From 1998 to 2006 223 VGS and S. bocis were isolated from blood in our institution. Erythromycin resistance was observed in 104 strains; 62 displayed cMLSb and 5 iMLSb phenotype. Sixteen strains were selected: 5 S. anginosus, 4 S. mitis, 3 S. constellatus, 2 S. bocis, and 1 S. salivarus cMLSb phenotype, 1 S. bocis i MLSb and 1 S. bovis cMLSb showing telithromycin resistance (MIC=4 mg/L). The erm(B) upstream region of these isolates was amplified from chromosomal DNA. The DNA sequences and the deduced amino acid sequences were compared to the upstream erm (B) region found in Tn1545 (accession no. X53632).

Results: Three different point mutations were detected in the leader peptide region compared to Tn1545: A138T was detected in 1 S. mitis cMLSb isolate resulting in N8Y substitution, G186A in 2 S. anginosus, 1 S. mitis and 1 S. bocis cMLSb isolates resulting in A25T substitution and in the telithromycin-resistant S. bocis isolate, G186A was detected resulting in a stop codon and a shorter leader peptide (16 amino acids) compared to Tn1545 (27 amino acids). A 24 pb duplication at the beginning (position 333) of the ORF of the erm (B) gene was also observed in the telithromycin resistant S. bocis strain. No mutations were detected in the remaining strains included.

Conclusions: Some of the mutations presented in this study are likely not involved in resistance and may represent heterogeneity in the erm (B) and its leader sequence among viridans group streptococci and Streptococcus bovis. However a shorter leader peptide and duplication in the ORF of the erm (B) gene were detected in a telithromycin resistant S. bocis strain and could be implicated in the ketolide resistance.

Analysis of alterations in the leader sequence of the erm(B) gene of different species of viridans group streptococci expressing MLSB resistance phenotype


Objectives: To study the antimicrobial susceptibility and prevalence of the different phenotypes of macrolide resistance in group A streptococci (GAS) isolated in Madrid, (Spain) in 2002–2007. The increase of the resistance and the changes in the implied mechanisms reduce the effectiveness of macrolides and clindamycin as an alternative treatment for GAS infections.

Methods: A total of 2380 isolates of Streptococcus pyogenes collected in the years 2002–2007 in Madrid, Spain were used. Antimicrobial susceptibility testing was performed using the agar diffusion method. Discs containing erythromycin and clindamycin were used to recognize the phenotypes of macrolide-lincosamide-streptogramin (MLS) resistance.

Results: Evolution of antimicrobial resistance is shown in figure 1. Although the overall level of macrolide resistance has remained stable in Spain (15–20%) in previous years, a rapid increase in the dominant phenotypes has been noted, with a sharp decrease in the MLS(B) phenotype paralleled by an increase in the M phenotype in the last years. In our study the peak was reached in 2004 showing 39% of the isolates resistance to erythromycin (81.9% M phenotype), with a continuous decrease since then to reach a minimum in 2008 (8.2%), although keeping the predominance of the M phenotype isolates (80.2%).
Conclusions: We have found a dramatic decrease of erythromycin resistance to GAS during the last years; this may be explained because of changes in the distribution of M phenotype in the community, due to the appearance of new epidemic clones as has been described recently.[1]

Reference(s)

P042 Bacteriophage-mediated transfer of erythromycin and tetracycline resistance genes among group A streptococci
L.A. Vitali*1, C.C. Di Luca, S. D’Ercole, D. Petrelli, M. Penna, S. Ripa (Camerino, IT)

Objectives: In *Streptococcus pyogenes* (Group A Streptococcus, GAS), genes conferring efflux-mediated erythromycin (ERY) resistance (mefA/msr) are carried by functional prophages and may be associated with the tetracycline (TET) resistance determinant tetO. We sought to demonstrate the “ex vivo” lyogenic transfer of ERY and TET resistance genes among GAS.

Methods: Forty-one ERY-susceptible GAS clinical isolates belonging to 12 different M-serotypes were used as recipients. The strain K56 (M-serotype 12) was used as a standard indicator to prepare lawns and as a model recipient, while the ERY- and TET-Resistant strain m46 (M-serotype 4) was used as the donor of the tetO-mefA phage (phi-m46). The donor was induced with 0.2mg/L mitomycin C. The culture supernatant was filtered, treated with DNase/RNase, concentrated by PEG/NaCl precipitation, and then added to cultures of the recipient strains. After incubation, these mixtures were either added to supplemented molten top agar and poured over BHI agar plates for the visualisation and counting of plaques or plated on BHI agar plus amoxicillin-Rstreptococci,irrespective of the duration of the amoxicillin course.

Results: The reference strain K56 and 85.4% (n=35) of the clinical isolates acquired ERY/TET resistance when infected with purified phi-m46. All the M1 (n = 7), M12 (n = 6), M75 (n = 2), M18 (n = 1), M94 (n = 1) and a fraction of the M3 (5/7), M4 (1/3), M5 (1/2), M6 (4/7) strains were lysogenised and converted to the ERY/TET resistant phenotype. No lysogenic clones were isolated in the case of the M77, M78 and M89 recipients. Only M12 strains and the totality of them were phi-m46-sensitive.

Conclusions: The erythromycin and tetracycline resistance determinants carried by phi-m46 can be efficiently transferred from a GAS strain to another by transduction. This is the first direct demonstration of phage-mediated horizontal resistance gene transfer in *S. pyogenes*. In the set of strains analyzed, an M-serotype dependent barrier to lysogenic transfer was not observed. The exclusive lysis susceptibility of the M12 strains indicates that GAS with this serotype may represent the main responsible for the spread of this phage and the associated antibiotic resistance genes in *S. pyogenes* global population.

P043 Amoxicillin resistance of oral streptococci in healthy patients undergoing tooth extraction

Objectives: The aims of this study were (i) to evaluate the presence of oral streptococci with reduced susceptibility to amoxicillin (ii) to identify to the species level the resistant isolates using phenotypic and genomic methods, and (iii) to follow up the evolution of the susceptibilities after amoxicillin therapy.

Methods: Thirty three healthy patients undergoing tooth extraction were randomly assigned in a double blind control study to a 7-day amoxicillin treatment or a 3-day amoxicillin + 4-day placebo treatment. The patients treated with any antibiotic in the 45 days before were excluded. Post-operative follow up was done 7 and 30 days after tooth extraction. Oral streptococcal flora was quantified on selective Columbia blood agar supplemented with 0 mg/L, 0.5 mg/L, 2 mg/L or 16 mg/L of amoxicillin. MICs were determined on streptococci by E-test® (Biomerieux). Amoxicillin-intermediate and resistant (amoxicillin-I and -R) strains were defined according to the recommendations of the CA-SFM. Identiﬁcations were ﬁrst determined by metabolic characters obtained on rapid ID32 STREP® gallery (Biomerieux). Final speciation was then determined by sequence analysis of the 16S rRNA and the sodA genes.

Results: A total of 43 amoxicillin-R (MIC > 16 mg/L) and 70 amoxicillin-I (0.5 < MIC < 16 mg/L) streptococcal strains were isolated. Most of the resistant strains were *Streptococcus oralis*, others were *Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus australis*, *Streptococcus parasanguinis* or *Streptococcus infantis*. Two and 28 patients were respectively colonised by amoxicillin-R or -I streptococci before treatment. Among the 22 patients followed up after treatment, all but two were colonised by amoxicillin-I and two by amoxicillin-R streptococci, irrespective of the duration of the amoxicillin course.

Conclusions: These results demonstrate both the presence of resistant oral streptococci in the commensal flora of healthy patients and their rapid selection by amoxicillin treatment. Given the role of oral streptococci in the acquisition of β-lactam resistance by virulent pneumococci, this study leads to promote short antibiotic course and to survey the antibiotic resistance among oral flora.

P044 Rapid detection of G2576T mutation conferring linezolid resistance in enterococci

Objectives: Resistance to linezolid has been described in clinical enterococci isolates and is mediated by a single nucleotide polymorphism (SNP) of guanine to thymidine at bp 2576 (G2576T) in the multiple rrl genes encoding 23S rRNA synthesis. The proportion of rrl genes with G2576T SNP (n=23) or the G2505A SNP (n=3). The assay was performed using a specific primer in which the first nucleotide was designed to produce a DNAmelting analysis point mutation (T) and the second nucleotide was designed to produce a mismatch in order to yield AS-PCR amplification. DNA melting analysis with fluorescent SYBR Green afforded detection of the PCR products on a Smart Cycler®.

Results: Perfect correlation was obtained between AS-PCR and reference sequencing method. All non enterococcal isolates, linezolid susceptible enterococcal isolates and linezolid resistant isolates containing a mutation at position 2505 were negative with our AS-PCR assay. The G2576T mutation responsible to linezolid resistance could be detected successfully by this method in enterococcal isolates. A good correlation was also found between linezolid MICs and the number of rrl genes carrying G2576T mutation.

Conclusion: The real-time AS-PCR assay for detection of G2576T mutation in Enterococcus carries an easy and rapid detection of linezolid
resistant strains of *E. faecalis* and *E. faecium*. This method is even able to identify a single mutated rrl gene which is known to increase the selection of linezolid resistant enterococci under linezolid pressure.

**[P945] Outbreak of multiple clones of linezolid- and vancomycin-resistant enterococci in the intensive care unit of a Greek university hospital**

E. Ntokou*, C. Stathopoulos, A. Ikonomidis, D. Maliris, E. Zakynthinos, A. Tsakris, A. Maniatis, S. Pournaras (Larissa, Athens, GR)

**Objectives:** Linezolid is often used for the treatment of infections due to vancomycin-resistant enterococci. During the last few years, linezolid-resistant enterococci are sporadically isolated from several hospitals where linezolid is overused. The current study investigated the dissemination and the resistance mechanisms of linezolid-resistant *Enterococcus faecium* and faecalis isolates causing an outbreak in a Greek University Hospital.

**Methods:** During the period January 2007 and December 2008, 23 linezolid-resistant *E. faecium* and faecalis clinical isolates were recovered from patients hospitalised in the intensive-care unit (ICU) of the University Hospital of Larissa, Greece. Isolates were screened by PCR using four overlapping sets of primers amplifying the total copy number of the 23S RNA subunit. Sequencing of the PCRs products revealed the mutations conferring linezolid resistance. The number of mutated copies was estimated from the presence and relative size of two different peaks in the mutated nucleotide position. The isolates were also screened by pulsed-field gel electrophoresis (PFGE) to determine the clonal clusters.

**Results:** Nine patients yielded linezolid-resistant *E. faecium* and five *E. faecalis* isolates. These isolates were multidrug resistant and recovered from bacteraemias in eight and from less significantly sites in the remaining six patients. The linezolid MICs of the 23 isolates varied from 24 to >256 µg/mL, while 11 isolates (47.8%) were co-resistant to vancomycin and 12 of 23 (52.1%) to teicoplanin. All of the isolates carried the commonly detected mutation G2576T. The amount of the mutated copies ranged from two to six and correlated with the dissemination and the resistance mechanisms of linezolid-resistant *E. faecium* isolates. Seventeen PRASEF isolates belonged to gdh allele type 12 and ST6. The three remaining PRASEF and all penicillin-susceptible isolates were not related to ST6. The seventeen ST6 isolates were genetically related as shown by PFGE (<5 band differences).

**Conclusion:** The emergence of PRASEF in North Denmark Region is mainly due to clonal spread of a specific lineage (ST6) that has acquired this atypical penicillin resistance phenotype. It is generally assumed that penicillin and ampicillin resistance are linked in enterococci, but the emergence of PRASEF underpins that this is not necessarily true. PRASEF constitutes a challenge to current guidelines for susceptibility testing and treatment of *E. faecalis* infections.

**References:**

1. H.C. Schoneheyder*, J. Larsen, L. Gudrupdassil (Aalborg, Frederiksborg, DK)

**Objective:** Penicillin resistance has remained rare in *E. faecalis* despite being frequent in *E. faecium*. In the last few years we have noted the emergence of penicillin-resistant ampicillin-susceptible *E. faecalis* (PRASEF) among blood culture isolates in North Denmark Region. The objective of this study was to elucidate the molecular epidemiology of PRASEF.

**Methods:** We identified 44 patients with *E. faecalis* bacteraemia in 2007 through a regional microbiology information system; clinical and epidemiological data were obtained from a regional bacteraemia register. Antibiotic susceptibility was determined by Etest using the Clinical and Laboratory Standard Institute (CLSI) breakpoints. We used pulsed-field gel electrophoresis (PFGE) to identify a single mutated rrl gene which is known to increase the selection of linezolid resistant enterococci under linezolid pressure. The number of mutated copies was estimated from the presence and relative size of two different peaks in the mutated nucleotide position. The isolates were also screened by pulsed-field gel electrophoresis (PFGE) to determine the clonal clusters.

**Results:** Nine patients yielded linezolid-resistant *E. faecium* and five *E. faecalis* isolates. These isolates were multidrug resistant and recovered from bacteraemias in eight and from less significantly sites in the remaining six patients. The linezolid MICs of the 23 isolates varied from 24 to >256 µg/mL, while 11 isolates (47.8%) were co-resistant to vancomycin and 12 of 23 (52.1%) to teicoplanin. All of the isolates carried the commonly detected mutation G2576T. The amount of the mutated copies ranged from two to six and correlated with the dissemination and the resistance mechanisms of linezolid-resistant *E. faecium* isolates. Seventeen PRASEF isolates belonged to gdh allele type 12 and ST6. The three remaining PRASEF and all penicillin-susceptible isolates were not related to ST6. The seventeen ST6 isolates were genetically related as shown by PFGE (<5 band differences).

**Conclusion:** The emergence of PRASEF in North Denmark Region is mainly due to clonal spread of a specific lineage (ST6) that has acquired this atypical penicillin resistance phenotype. It is generally assumed that penicillin and ampicillin resistance are linked in enterococci, but the emergence of PRASEF underpins that this is not necessarily true. PRASEF constitutes a challenge to current guidelines for susceptibility testing and treatment of *E. faecalis* infections.

**References:**

1. C. Novais, A. Fontes, F. Baquero, L.V. Peixe, T.M. Coque (Porto, PT, Madrid, ES)

**Objective:** Conjugal transposons (CTn) have contributed to the spread of tetracycline (Te) resistance and have been associated with tetM and rarely with tetS. The latter was recently described in EfcTn1 from an *E. faecium* (Efm) of a primate. We evaluated the frequency of EfcTn1 in enterococci from different settings and characterised its molecular structure.

**Methods:** Enterococci (n = 616) from several Portuguese sources (animals or animal environment-260, healthy human (HH)-125, sewage/river-54, clinical samples (CS)-102 from 1999–2006) and 97 enterococci representative of HreCC nosocomial strains from other 19 countries of 5 continents (1986–2006) were screened for the presence of tet genes (M, O, L, S, K), integrases, excisionases, transposases or tet genes. The latter was recently described in EfcTn1 from an *E. faecium* (Efm) of a primate. We evaluated the frequency of EfcTn1 in enterococci from different settings and characterised its molecular structure.

**Results:** Twenty cases of PRASEF bacteraemia, 15 of which were nosocomial. At the time of diagnosis patients were admitted to 7 hospitals with different types of infection (Table). PRASEF isolates had penicillin MICs ≥16 µg/mL and ampicillin MICs ≤4 µg/mL. No β-lactamase activity was detected. Two PRASEF isolates were resistant to imipenem (MICs ≥16 µg/mL). High-level resistance to gentamicin was common (17/20 isolates). All isolates were susceptible to vancomycin. Seventeen PRASEF isolates belonged to gdh allele type 12 and ST6. The three remaining PRASEF and all penicillin-susceptible isolates were not related to ST6. The seventeen ST6 isolates were genetically related as shown by PFGE (<5 band differences).

**Conclusion:** The emergence of PRASEF in North Denmark Region is mainly due to clonal spread of a specific lineage (ST6) that has acquired this atypical penicillin resistance phenotype. It is generally assumed that penicillin and ampicillin resistance are linked in enterococci, but the emergence of PRASEF underpins that this is not necessarily true. PRASEF constitutes a challenge to current guidelines for susceptibility testing and treatment of *E. faecalis* infections.

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1. C. Novais, A. Fontes, F. Baquero, L.V. Peixe, T.M. Coque (Porto, PT, Madrid, ES)
Portuguese healthy human volunteers are reservoirs of conjugative transposons associated with tetracycline and/or erythromycin resistance


Objectives: Conjugative transposons (CTn) have contributed to the spread of tetracycline (Tc) and erythromycin (Ery) resistance and they might influence the adaptation of enterococci to different environments. Diversity of Gram positive CTn was analyzed among enterococci from Portuguese healthy human volunteers (HV).

Methods: We studied 125 enterococci (61 E. faecalis-Ef, 51 E. faecium-Efm, 13 E. faecalis spp-Ef-Efm) from the faeces of 80 HV obtained in the Centre and North of Portugal in 2001. Resistance to Te and Er was observed in 74% of the isolates studied each. Resistance genes were analyzed by PCR (tetM, tetO, tetS, tetT, ermA). The presence of known Gram positive CTn and other Tn frequently associated with them were screened by a multiplex PCRs for detecting integrases, excisionases, transposases and relaxases of Te916, Te917, Te939, Te5398, Ef70, CW459TetM, or Tk3586.

Results: Tet genes were found among isolates resistant (n = 93), moderately resistant (n = 8) or susceptible (n = 24) to Te. We found tetM, tetL and tetS among Ef (75%, 26%, 7%), Efm (69%, 45%, 0%) and Ep (62%, 33%, 0%). Occurrence of int/xisTe916, tspA/tmpKTe917, orf298/Tn5398 or int/xisEfCTn1 was higher for Ef than for Efm or Ep (69%/25%/46%; 15%/8%/0%; 52%/25%/46%; 8%/0%/0% respectively). tndXTn3597 was more common among Ef (18%) than Efm (2%). IntCW459TeM was detected for the first time in enterococci and was observed in 4 Ep, 3 Ef and 1 Efm. Among isolates harbouring CTn, 57% presented one and 43% or more of these genetic elements (int/xisTe916+orf298/Tn5398−24%; int/xisTe916+tmpA/tmpKTe917−6%; int/xisTe916+tmpA/tmpKTe917+orf298/Tn5398−3%; int/xisTe916+tmpA/tmpKTe917+orf298/Tn5398+ int/xisEfCTn1−2%; 8 other combinations with 1 isolate each).

Conclusion: Portuguese HV are important reservoirs of Gram positive Tns associated with Te and Ery resistance. Some Clostridium sp typical genetic elements were isolated for the first time in enterococci from this setting (CW459TeM, Tn5398) and their high occurrence suggest frequent mobilisation among different bacterial genera.

Characterisation of Gram-positive conjugative transposons among international epidemic vancomycin-resistant isolates of Enterococcus faecalis and Enterococcus faecium

C. Nocais*, A. R. Freitas, M.V. Francia, L.V. Peixe, F. Baquero, T.M. Coque (Porto, PT; Santander, Madrid, ES)

Objectives: Conjugative transposons (CTn) contributed to the spread of antibiotic resistance in enterococci (tetracycline, TeR, erythromycin- ErR and vancomycin-VcR). Diversity of all known Gram positive CTns was analyzed by a multiplex PCR in clinical vancomycin-resistant enterococcal strains causing hospital outbreaks in different continents.

Methods: We studied 63 E. faecium (Ef) and 32 E. faecalis (Ef) isolates from different patients of 20 countries (Portugal, Spain, France, Italy, UK, Poland, Germany Hungary, Cyprus, Greece, Serbia, Norway, Netherlands, Denmark, USA, Chile, Brasil, Argentina, Australia, Singapore) collected from 1986 to 2006. They are representative isolates causing well characterised clinical outbreaks isolates, most of them belonging to High Risk Clonal Complexes (HirCC) of Ef (CC2, CC9, CC87) and Ef70 (CC17). All but two isolates (Ef70) were resistant to vancomycin (vanA-68%; vanB-25%; vanG-1%). Characterisation of tet (tetM, tetO, tetL, tetS, tetT) and ermB genes was performed by PCR. The presence of integrases/excisionases from Gram positive CTns or other Tn associated to them (Te916, Te917, Tn5397, Tn3598, Ef70, CW459TetM, Tn5386) was screened by multiplex PCR assays and further sequencing. Specificity of the method was proved by using specific control strains and sequencing PCR products.

Results: We identify tetM, tetL and tetS in Ef (69%, 41%, 9%) and Ef70 (38%, 21%, 0%), ermA was also detected in Ef (100%) and Ef70 (87%). Occurrence of int/xisTe916/Tn5145, orf298/Tn5398, tspM/mpKTe917 was higher among Ef [47%-10 countries (c), 72%-6c, 44%-6c] than Ef (13%-7c, 18%-6c, 13%-4c). Conversely, intCW459tetM was more common among Ef than Efm (30%-8c and 19%-4c, respectively). tndXTn3597 was only detected in 2 Ef and 1 Efm from Spain and Portugal and int/xisEfCTn1 in two Ef strains from USA. A high rate of isolates (35%) presented more than one Tn.

Conclusion: A diversity of Gram positive CTns was observed among nosocomial epidemic strains of enterococci from different continents. Beside antibiotic resistance dissemination, CTn might be involved in recombinatorial events and/or spread of other genetic elements favouring the maintenance of HirCC strains in the hospital setting. This is the first description of IntCW459TeM in enterococci, and of int/xisEfCTn1 among enterococcal strains associated with the hospital environment.

Prospective study of Corynebacteria other than Corynebacterium diphtheriae in clinical specimens: identification, clinical relevance and antibiotic susceptibility

A. Ferjani, S. Mhalla, M. Marzouk, N. Hammuchi, J. Bonkadda* (Sousse, TN)

Objectives: Corynebacteria other than Corynebacterium diphtheriae have increasingly been reported as opportunistic pathogens in nosocomial infections. Moreover, new species isolated from humans have recently been described. The purpose of this study was to evaluate the distribution of corynebacteria species other than Corynebacterium diphtheriae, using currently available identification methods. The clinical relevance of the isolates was assessed and the antibiotic susceptibility was determined.

Materials and Methods: The isolates were collected over a period of 2 years (January 2007 to December 2008), from different human specimens coming from different departments. Strains were identified according to metabolic and biochemical characters (Api Coryne, BioMérieux). Antimicrobial susceptibility tests were carried out by MIC determination using the E-test (AB BIODISK). The susceptibility breakpoint concentrations were as recommended by the Clinical and Laboratory Standards Institute (CLSI).

Results: During the period of study, 33 strains were collected and included in this study. C. striatum and C. macrogeloides were mainly isolated, 39.4% and 33.3% respectively. The majority of isolates came from hospitalised patients (79%), essentially from ORL department. six patients (18%) had less than 15 years, 7 (21.1%) were immunocompromized and the sex ratio was of 0.4. Of the 33 strains, 8 (24.2%) came from ear infection specimens, 6 (18%) from biomaternal samples from thorax drains (3/6) and 6 (18%) from wounds cultures. Urinary tract specimens represented 4 (12%) and lower respiratory tract specimens 3 (9%). The high rate of resistance with high MICs was showed for penicillins (42%), erythromycin (60%) and clindamycin (60%). Thirty-three percent
(11 isolates) were resistant to fluoroquinolones and 36% to cotrimoxazole (12 strains). No resistance was noted to glycopeptides and generally the activity of teicoplanin was superior (MIC lower) to that of vancomycin. Among the 13 multiresistant strains (39%), 7 (53%) were C. macginleyi. 4 (30%) were C. striatum and one strain of each was C. jekesium and C. pseudoophterium.

Conclusions: Corynebacteria other than Corynebacterium diphtheriae are of exceptional cause of human infections, implicated essentially in ear and wound infections. C. striatum and C. macginleyi were mainly isolated. High levels of resistance were noted for penicillins, erythromycin and clindamycin. Glycopeptides are the antibiotics of choice.

Sepsis, bacteraemia and endocarditis

Objective: To describe our experience in treating infective endocarditis (IE) with outpatient parenteral antibiotic therapy (OPAT), and to identify new groups of patients with IE who may qualify for OPAT.

Methods: Following institutional review board approval, patients discharged from Baystate Medical Centre with IE documented by modified Duke criteria treated with OPAT were identified. Data obtained included organisms implicated, endocardial surface involvement, emergent surgical intervention, indications of clinical stability, percentage of total therapy rendered after hospitalisation, and one year follow-up. Pairwise comparisons of clinical groups were conducted using the Wilcoxon rank-sum test. For these comparisons, medians are used. Pairwise comparisons of clinical groups were conducted using the Wilcoxon rank-sum test. For these comparisons, medians are used.

Results: Forty-three patients met criteria. Thirty-five percent were infected with Staphylococci; 40% harboured streptococci or Enterococci. Native valves and left-sided valves each constituted approximately 75% of total. All patients received ≥ 4 weeks of therapy, with ≥ 66% of total treatment rendered after hospital discharge. The table provides further pathogen-specific data. A median of 7 days of haemodynamic stability and negative blood cultures occurred prior to discharge. After one year, no patients died from IE. Twenty-three percent were hospitalised during OPAT from intravenous catheter, antibiotic, or other complications, none for direct complications of IE.

Conclusions: Outpatient parenteral antibiotic therapy for infective endocarditis can be safely utilised, and at least 66% of care can be given in this manner. Our investigation provides enhanced data for employing OPAT for IE caused by Staphylococci and left-sided cardiac infections, and also provides favourable outcome data one year after treatment.

Distribution of patients by infecting organism, infected endocardial surface, requirement for surgery, total treatment duration and OPAT

<table>
<thead>
<tr>
<th>Infecting organism</th>
<th>Number of patients (N=43)</th>
<th>% Left-sided disease (N=33)</th>
<th>% Required emergent surgery (N=12)</th>
<th>Duration of OPAT treatment (weeks)</th>
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<tr>
<td>Vindan streptococci</td>
<td>8 (18%)</td>
<td>1 (8%)</td>
<td>2 (17%)</td>
<td>4 (78%)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>5 (12%)</td>
<td>2 (15%)</td>
<td>0 (0%)</td>
<td>6 (120%)</td>
</tr>
<tr>
<td>Methicillin susceptible Staphylococcus aureus</td>
<td>9 (21%)</td>
<td>8 (88%)</td>
<td>2 (22%)</td>
<td>6 (80%)</td>
</tr>
<tr>
<td>Methicillin resistant Staphylococcus aureus</td>
<td>1 (0%)</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>90 (1300)</td>
</tr>
<tr>
<td>Sphingobacterium non aureus</td>
<td>5 (12%)</td>
<td>3 (60%)</td>
<td>0 (0%)</td>
<td>10 (200)</td>
</tr>
<tr>
<td>Chlamydia sp.</td>
<td>9 (21%)</td>
<td>7 (77%)</td>
<td>2 (22%)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Other streptococci</td>
<td>4 (9%)</td>
<td>3 (75%)</td>
<td>0 (0%)</td>
<td>4 (60%)</td>
</tr>
<tr>
<td>HACEK</td>
<td>1 (2%)</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>6 (90)</td>
</tr>
<tr>
<td>Peptostreptococci</td>
<td>1 (2%)</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>6 (90)</td>
</tr>
</tbody>
</table>

*1 patient lost to follow-up.

Outcome of empiric versus targeted antibiotic therapy in infective endocarditis, Medellin, Colombia. Study of 120 cases over a 20-year period

C. Bastamante, S.M. Gil, G.M. Montoya, M. Zuluaga, N. Lopez, G. Franco, J.C. Gonzalez* (Medellin, CO)

Objectives: Despite current medical developments, mortality of infective endocarditis (IE) reaches 25% and without treatment, IE can be lethal. Although one of the most crucial factors to improve the outcome is the use of targeted antibiotic regimen guided by bacteriological results, the microbiological diagnosis is not always possible and empiric treatment is required. The outcome differences between both approaches are not yet extensively evaluated in Latin America. The aim of this study was to compare the outcome (complications and death) between patients with proven diagnosis of IE according to Duke Criteria that received targeted or empiric antibiotic therapy.

Methods: Retrospective evaluation of medical records of hospitalised patients attended between 1988 and 2008 in a 140 bed specialised cardiovascular clinic, was performed. The epidemiological features and clinical outcome were assessed in both groups. The statistical analysis included Pearson’s square Chi test.

Results: In 20 years, 120 adult patients fulfilled IE Duke criteria. 96 (80%) received targeted antibiotic therapy and 24 (20%) received empiric treatment according to American Heart Association (AHA) guidelines, adapted to known local antibiotic resistance profiles. 28 (23.3%) patients had a valvular prosthesis, among them 24 received targeted treatment and 4 empiric treatment. Surgery was also needed in 56 patients of targeted treatment group and 19 of empiric treatment group (57.7% and 79.3%, respectively). Overall, no differences in hospital mortality (targeted treatment 19.6% vs. empiric treatment 20.8%) or complications (arrhythmias, abscesses, rupture of tendinous cords, valvular perforation or prolapse, acute cardiac failure, acute renal failure, sepsis, need of valvular surgery, surgical reintervention) were observed between empiric versus targeted treatment.

Conclusions: Interestingly, no differences in mortality and complications were found between targeted and empiric treatment. It could be explained by a close adherence to antibiotic guidelines and the knowledge of nosocomial infections profile. Remarkable, most IE cases were targeted treated indicating accurate microbiological methods and no previous use of antibiotics. Since the mortality and complications of empiric treatment cases were similar to targeted treatment, we emphasize the importance of following antibiotic guidelines and the determination of epidemiological patterns in each centre.

Epidemiology, clinical and microbiological features of infectious endocarditis: a review of 54 cases

D. Kofieridis, M. Christofaki*, A. Valachis, C. Mathoos, D. Dimopoulou, I. Aristidou, A. Christidou, G. Samonis (Heraklion Crete, GR)

Objectives: The purpose of the study was to investigate the aetiologies, risk factors, clinical features and outcome of infective endocarditis (IE) in the area of Crete.

Methods: The medical records of all patients hospitalised at the University Hospital of Heraklion, Crete, Greece, diagnosed with IE from 1993 to 2007 were retrospectively reviewed. All patients who met the modified Duke criteria for definite IE, were included.

Results: Fifty four cases of definite IE occurring in the same number of patients were evaluated. The median age of patients was 60 years (range 46–74). There were 35 males (64%). Transthoracic echocardiography (TTE) was performed in 37 patients (68%), and transesophageal (TEE) in 19 (35%). Findings consistent with endocarditis were found in 32 (86%) out of 37 TTE and in 16 (84%) out of 19 TEE. Most cases [41 patients (76%)] were native valve IE. Predisposing conditions were evident in 40 patients (74%) and included prosthetic valve [13 patients (24%), injected drug use [1 (1.8%)], mitral valve prolapse [6 (11%)], poor dental hygiene [3 (5%)], diabetes [10 (18.5%)] and HIV infection [1 (1.8%)]. The mitral valve was affected in 26 patients
(48%), the aortic in 22 (40%), while 6 (11%) had both valves affected. Blood cultures were negative in 8 cases (15%). The leading causative microorganism was S. aureus isolated in 15 cases (28%), followed by coagulase-negative staphylococci in 12 (22%), viridans streptococci in 9 (16.6%) and E. faecalis in 5 (9%). A number of rare and difficult to treat microorganisms had been identified such as G. morbillorum in 2 (4%) cases, S. lugdunensis in 2 (4%), Brucella spp in 1 (2%), and S. pneumoniae in 1 (2%). One patient had positive serologic test for C. burnettii (2%). All patients received antimicrobial treatment on empirical basis, which was proven appropriate in 43 patients (93%) based on the results of blood cultures. Seven patients (12%) had surgical treatment. In-hospital mortality reached 15% (8 patients).

Conclusions: Staphylococcus and Streptococcus spp remained the most common aetiologic agents of IE. However, the presence of uncommon and/or difficult to treat pathogens raise concern that appropriate prophylaxis and empirical treatment may be more complicated than believed in the past. Furthermore serology for C. burnettii should be included in the diagnostic work-up in endemic areas.

**P954** 120 blood cultures negatives endocarditis in southern Spain


**Objectives:** To know the clinical features and possible aetiologic agents of the blood culture negative endocarditis (BCNE).

**Methods:** Descriptive cross sectional study in a series of patients with Infectious Endocarditis (IE) diagnosed from 1986 to 2007 in seven second and tertiary hospitals in the south of Spain.

**Results:** 120 (14.8%) of 359 cases of IE included were categorised as BCNE affecting in 71.7% and 28.3% to native and prosthetic valves respectively. The mean age was 60 ± 12 years. Seventy eight (62.5%) patients were male. The most affected valve was aortic in 49.2%, mitral in 35%. An nosocomial endocarditis was found in 8.3% and previous manipulation in 26 cases (21.6%). The clinical symptoms until diagnosis were 68±126 days. The symptoms were fever (93.1%), new murmur (67.5%), chills (45%), splenomegaly (45%) and hepatomegaly (45%). In 14.2% we detected vascular phenomena and immunological phenomena were present in 15%.

Antibiotics were used previously in 67 (55.8%) patients, serologic test were positive in 24 cases (20%) and valvular cultures in 12 (10%). In 83 cases (69.2%) we didn’t find germs, in 19 (15.8%) Coxsella burnetti, in 4 (3.3%) Brucella, in 4 (3.3%) Fusiss, in 2 (1.7%) S. coagulase negative, in 2 (1.7%) S. aureus, in 1 (0.8%) Bartonella and in 1 (0.8%) Mycoplasma.

Transthoracic echocardiogram (TTE) alone was performed in 45.5%, Transoesophageal echocardiogram (TEE) in 4.2% and TTE + TEE in 45.8%. The TEE was diagnostic in 85% and the TEE only in 69%. Complications seen in Echo or Surgery were valvular rupture (12.6%) and abscess (10%). Surgery (60%) was undertaken in 49.2% during the hospital admission and in 10.8% cases later. Congestive heart failure was the main reason for surgery (67%), valvular disfunction (20%), sepsis (9%) and abscess (2%). The global mortality ratio was 20%.

**Conclusions:** 1) Antibiotics taken before a IE diagnosed is the main factor for the negativity of the blood culture. 2) Serologic tests for Brucella, C. burnetti and Bartonella might be considered in BCNE mainly in endemic areas. 3) The histologic and microbiologic examination of the valves after the surgery is so much important to identify the aetiologic agent. 4) Molecular techniques may be an interesting alternative diagnostic test for IE caused by bacteria that usually give a negative blood culture. 5) Negative blood cultures endocarditis presents a high surgery percentage with similar mortality than positives blood culture endocarditis.
Heart surgery was carried out in 33 pts (27%) before finishing antibiotic treatment. Thirty-one patients died due to endocarditis (25%).

**Conclusion:** The ascertained incidence of IE is lower than expected, probably because of insufficient availability of good echocardiography in several hospitals. We confirmed the new trends in IE characteristics: raising men/women ratio, raising frequency of aortic valve involvement, prevailing staphylococcal aetiology. High rate of culture-negative endocarditis (CNE) documents incorrect antibiotics prescription habits. We feel that most CNE cases were caused by antibiotic-sensitive streptococci.

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**P955** The incidence of sepsis in a large Dutch University hospital: CRP and the SIRS criteria

T.T.N. Le*, H.J van Leeuwen, E.E. Mattison, J.A.G. van Strijp, J. Verhoef (Utrecht, Arnhem, NL)

**Objectives:** In 1991, consensus guidelines were established for diagnosis of sepsis. Sepsis is defined as an infection with symptoms of systemic inflammatory response syndrome (SIRS). This sepsis definition was reviewed in 2001 and has been found to be sufficient only with the addition of more possible symptoms and signs of SIRS and biomarkers like C-reactive protein (CRP), although it was premature to use biomarkers to diagnose sepsis. We conducted a prospective, observational study on the epidemiology of sepsis at the University Medical Center Utrecht. We evaluated the value of CRP in addition to the SIRS criteria to differentiate sepsis from SIRS or seriously ill patients.

**Methods:** At the University Medical Center Utrecht, all patients from whom blood cultures were drawn were evaluated between October 2004 till September 2005 for having sepsis. All patients with a possible infection and who met ≥2 of the SIRS criteria or patients who did not met the SIRS criteria but who were seriously ill (altered mental status, organ failure, hypotension) were enrolled in the study. Sepsis and SIRS patients (seriously ill patients without infection) were divided into three groups: patients without organ failure, patients with organ failure and patients with shock. SIRS and sepsis patients in the same category were compared with respect to the SIRS criteria and CRP. Area under the curve (AUC) in ROC curves were calculated (AUC > 0.8 was considered discriminatory).

**Results:** 6203 blood cultures were drawn from 2197 patients. Based on the above mentioned criteria 2025 episodes in 1676 possible sepsis patients were identified after a first evaluation. After a further second evaluation of these episodes with knowledge of the culture results and clinical course 998 patients with definitive sepsis and 408 patients were identified after a further second evaluation. On the above mentioned criteria 2025 episodes in 1676 possible sepsis patients were identified after a first evaluation. After a further second evaluation of these episodes with knowledge of the culture results and clinical course 998 patients with definitive sepsis and 408 patients were identified after a further second evaluation. On the above mentioned criteria 2025 episodes in 1676 possible sepsis patients were identified after a first evaluation. After a further second evaluation of these episodes with knowledge of the culture results and clinical course 998 patients with definitive sepsis and 408 patients were identified after a further second evaluation.

**Conclusion:** Patients with severe sepsis and bacteremia do not seem to have a different immune profile than patients without bacteremia. Bacteremia seems to influence LOS but not the final outcome.

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**P958** The effect of bacteremia on the immune response and outcome of patients with severe sepsis

L. Leonidou*, A. Moszaki, A. Georgakopoulou, V. Kyriazopoulou, H.P. Bassaris, C.A. Gogos (Patras, GR)

**Objectives:** The aim of the present study was to examine the levels of pro and anti-inflammatory cytokines and outcome in patients with severe sepsis and bacteremia.

**Patients and Methods:** The study included 56 patients with severe sepsis. The patients were divided into two groups according to the presence or absence of positive blood cultures: patients with bacteremia (group B, n = 17) and non-bacteremic patients (group NB, n = 39). Severe sepsis was defined as the presence of confirmed infection and ≥2 of the following criteria: (a) a temperature >38°C or <36°C, (b) heart rate >90 beats/min, (c) respiratory rate of >20 breaths/min, (d) WBC >12,000 or <4,000 cells/mm3 plus at least one organ dysfunction indicated by the following: (a) hypotension, (b) PaO2 <75 mmHg without evidence of primary respiratory tract disease, (c) pH <7.3 or a base deficit of >5 meq/liter, (d) urine output <30 ml/h, (e) liver dysfunction, (f) acute alteration of mental status, or (g) DIC. The severity of sepsis was classified by the sepsis-related organ failure assessment score (SOFA). Levels of the pro-inflammatory cytokines TNF-α and IL-6 and anti-inflammatory cytokine IL-10, as well as TGF-β1 were measured within 24 hours after admission (mean±SEM, pg/ml).

**Results:** The most common pathogen was E. coli (5/17, 29%). Other pathogens isolated were: S. aureus (3/17), P. aeruginosa (2/17), Enterococcus spp. (1/13), E. cloacae (1/13), S. pneumoniae (2/13), S. viridans (1/17), S. epidermidis (2/17). Six out of 17 patients (35.2%) with bacteremia and 10 out of 45 (22.2%) patients without bacteremia died (difference not significant). Group B had similar levels of IL-6 with group A (121.3±32.9 vs 108.4±13.9, p=0.9) and similar levels of IL-10 (10.2±4.2 vs 33.1±12.1, p=0.7). There was no difference in TGF-β1 (22.7±3.2 vs 21.6±1.9, p=0.4) and TNF-α levels (44.4±10.9 vs 34.1±5.1, p=0.3) between the two groups. Patients of both groups had a similar SOFA score (4.4±1.0 vs 3.4±0.5) and CRP levels (14.6±2.4 vs 15.2±1.7, p=0.8). Patients with bacteremia stayed more days in hospital than patients without. (Length of hospital stay (LOS): 12.5±2.9 vs 7.7±0.8 days, p=0.03)

**Conclusion:** Patients with severe sepsis and bacteremia do not seem to have a different immune profile than patients without bacteremia. Bacteremia seems to influence LOS but not the final outcome.

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**P959** Analysis of immune competent cells following major and minor visceral surgery provides insight into origin of postoperative sepsis


**Objectives:** Abdominal surgery is frequently followed by immune dysfunction which usually lasts for several days. Septic complications in this stadium of postoperative immune dysfunction result in increased mortality. Consequently we previously categorised sepsis into type A (spontaneously acquired) and type B, which is acquired postoperatively and associated with a higher mortality rate.

**Methods:** We analyzed expression of HLA-DR on monocytes of 118 patients by flow cytometry prior to and 24, 48 and 72 hours after surgery. For statistical analysis we used Mann-Whitney-Test, p was considered significant if <0.05.

**Results:** We hereby describe a significant reduced HLA-DR expression on monocytes following major surgery compared to minor surgery. Both groups differ from extent of trauma, blood loss and duration of operation. 24h postoperatively, we detected decreased expression of HLA-DR on circulating monocytes and therefore reduced immune function (p < 0.0005) following major compared to minor surgery. These differences were constant over a period of three days. We observed a significant reduction of HLA-DR expression if the operation required more than 2.5 hours.

Furthermore we analyzed HLA-DR expression in correlation with the incidence of postoperative sepsis. We compared monocytes of patients with severe postoperative sepsis to monocytes of a non-complicated postoperative progress. No differences concerning pre-operative HLA-DR expression could be displayed between both groups. 24 hours after surgery both levels decreased to 25% of initial value. Monocytes of patients with an uncomplicated postoperative course showed regeneration of HLA-DR levels within the first three days. In contrast, patients with septic complications had prolonged suppressed levels of HLA-DR expression resulting in significant differences between both groups.
Prognostic factors of community-acquired severe sepsis and septic shock


Objective: We sought to determine the independent risk factors on mortality in community acquired bacteremia patients with severe sepsis and septic shock.

Methods: A single-site prospective cohort study in a medical-surgical ICU in an academic tertiary care centre. Seventy patients with community acquired bacteremia severe sepsis and septic shock were identified. Clinical, microbiologic and laboratory parameters were compared between hospital survivors and hospital death.

Results: The global mortality rate was 50%, 52.8% in septic shock and 41.2% in severe sepsis. One or more comorbidities was present in 64.3% of patients. The most commonly identified bloodstream pathogen was Escherichia coli (24.3%). Gram-positive microorganisms were isolated in 51.4% of blood cultures. The proportion of patients receiving inadequate antimicrobial treatment was 7.1%. By univariate analysis, age, APACHE II score, 3 or more organ dysfunctions, and albumin, but neither inadequate empirical antimicrobial treatment nor microbiologic characteristics nor site of infection, differed significantly between survivors and non-survivors. APACHE II (OR: 1.19; 95%CI: 1.08−1.31) and albumin (OR: 0.13; 95%CI: 0.04−0.44) were independent risk factors associated with global mortality in logistic regression analysis.

Conclusions: APACHE II score and low levels of albumin were associated with increased mortality. Our results support that inadequate empirical antimicrobial treatment is not a significant factor to outcome in community acquired severe sepsis and septic shock in standard clinical setting.

Risk factors for and prognosis of sepsis secondary to severe or complicated pyelonephritis

N.N. Macías* (Malaga, ES)

Objectives: The aim was to identify the possible risk factors associated with the prognosis of severe sepsis in patients with severe or complicated pyelonephritis.

Methods: Retrospective, descriptive study including 1101 patients older than 14 years with severe or complicated pyelonephritis who were admitted in Internal Medicine or Infectious Diseases wards of a tertiary hospital between January 1997 and December 2007. Three hundred and one of them (28.1%) developed severe sepsis according to International Sepsis Definitions Conference criteria. All patients were treated and followed homogenously according to a previously defined protocol. Demographic, clinical, analytical, microbiological and sonographic variables were compared between patients with and without severe sepsis.

Results: Of the 1001 patients, 335 (30.4%) were men and 766 (69.6%) women. The mean age was 55.6±12.0 years (range, 14−97 years). Five hundred and eighty eight patients had one or more structural or functional urinary tract disorders. In multivariate analysis, male sex (OR: 1.03; C195%: 1.02−1.04), recent urinary instrumentation (OR: 3.64; C195%: 1.74−7.63), nosocomial acquisition, thrombocytopenia (OR: 2.57; C195%: 1.66−3.96), creatinine (OR: 1.41; C195%: 1.21−1.66), age (OR: 1.66; C195%: 1.15−2.39), positive blood cultures (OR: 6.34; C195%: 3.6−11), and grade III/IV sonographic ectasia (OR: 1.94; C195%: 1.38−3.06) were associated to severe sepsis.

Conclusions: The incidence of severe sepsis in complicated or severe pyelonephritis is high. There are demographic, clinical, analytical, microbiological and sonographic data independently associated with urinary severe sepsis. Patients with complicated pyelonephritis and severe sepsis have a considerable attributable mortality.

A circulating factor of patients with septic shock stimulates release of angiopeptin-2 by human monocytes

H. Kramidioti, A. Kotsuki, M. Moukaroudi, D. Plachouras, A. Savva, E.J. Giannarelli-Bourboulis* (Athens, GR)

Objectives: Angiopetinant (Ang)-2 was shown to circulate in high levels in the serum of patients with septic shock (Orfano S, et al. Crit Care Med 2007; 35: 199). The present study attempted to unravel the existence of any factor in serum stimulating the production of Ang-2 by human monocytes.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from 14 healthy volunteers after gradient centrifugation over Ficoll. Serum was sampled from four patients with sepsis and 10 patients with septic shock on day 1 of diagnosis (ACCP/SCCM 1992). PBMCs were stimulated for 24 hours at a density of 5×10^6/ml with 50% of patients’ serum in the absence or presence of 10ng/ml of lipopolyssaccharide (LPS) of Escherichia coli O155:H5, of 3 microM of the MAP kinase inhibitor SB203580 and of 1 microg/ml of E. coli TLR-4 antagonist (aTLR4). Ang-2 was estimated in supernatants by an enzyme immunoassay after adjustment for serum levels. Then 1×10^6/ml of PBMCs were stimulated for four hours either with medium or serum of patients with septic shock or with 10ng/ml of LPS. RNA was extracted after trizol and chloroform treatment and cDNA was synthesized. Expression of Ang-2 was estimated by real-time-PCR against the expression of β-2-microglobulin as a reference gene.
Results: Ang-2 production after cell stimulation is shown in Figure 1 (results as means ± SE, pg/ml). Median rate of Ang-2 gene expression after serum stimulation was 13.7, that after LPS stimulation was 5.9.

Conclusions: A circulating factor exists in serum of patients with septic syndrome stimulating release of Ang-2 by human PBMCs. Its concentrations are greater in septic shock than in sepsis. Release is antagonised by LPS and the MAP kinase pathway and is mediated, at least in part, by stimulation of gene expression.

**P964** The use of the Pneumoslide test in blood cultures and its relation to capsular serotype

*WW Chan*, M. Loxgren, G.J. Tyrrell (Edmonton, CA)

Objectives: The BBL Pneumoslide is an agglutination test for the rapid identification of *Streptococcus pneumoniae*, consisting of latex beads coated with polyvalent antiserum which reacts with pneumococcal capsular antigens. When used directly on positive blood cultures, it can yield an rapid diagnosis of pneumococcal bacteraemia, but the data on its performance in this setting is limited. Because it is based on antibodies targeting the capsular antigen, it is likely that test sensitivity would differ between serotypes. Our objective was to assess its performance in blood cultures at our centre, and evaluate the variation between serotypes.

Methods: A retrospective review was conducted of the blood cultures processed at the Department of Medical Microbiology, at the University of Alberta Hospital over an 18-month period. All blood cultures flagged as positive by the Bactec 9240 system and shown to be Gram-positive cocci in chains/pairs were tested with the Pneumoslide. Identification was confirmed using routine biochemical methods, including optochin susceptibility and bile solubility. Capsular serotyping was done by the Quellung method.

Results: 41,528 blood cultures were reviewed, with 3038 positives, and 290 yielding a Gram stain of Gram-positive cocci arranged in pairs or chains. Of these, 73 were ultimately identified as *S. pneumoniae*, and 55 were positive by the Pneumoslide assay. The 217 that were Gram-positive cocci other than *S. pneumoniae* registered 3 positive results. This corresponded with a sensitivity of 75.3% and a specificity of 98.6%, corresponding with a positive-predictive value of 94.8% and a negative-predictive value of 92.2%. When analyzed by serotypes, certain serotypes (7F, 15B, 33A, 38) were negative every time. The Pneumoslide was 50% accurate for serotypes 5 and 12F, 86% accurate for 22F, and 100% accurate for the remainder of the serotypes isolated from positive blood cultures.

Conclusion: The Pneumoslide test, when used directly on positive blood cultures, is useful in the rapid diagnosis of *Streptococcus pneumoniae*. It has a high specificity, with somewhat lower sensitivity, correlating with good positive and negative predictive values. Sensitivity varies by capsular serotype, and thus the local epidemiology of *S. pneumoniae* will alter the utility of the test.

**P965** Sentinel survey of typhoid prevalence among febrile patients attending clinics in Bushenyi district of Uganda

E Agwa*, L. Wambua, L. Titus (Ishaka, Uganda)

Background/Objective: Recent country data on the geographical distribution of typhoid indicates that western districts of Uganda were among the most affected. We therefore surveyed the prevalence of typhoid among febrile patients attending clinics in Bushenyi district and suggested intervention strategies to clamp down typhoid cases.

Methods: Six hundred and eighty seven blood samples were collected aseptically and analyzed using standard Widal serological Slide agglutination and tube dilution of somatic and flagella antigens of *Salmonella enterica* serotype Typhi. Chi-square test (p = 0.05; 0.01) was used to test for statistical significance of age, sex, socio-economic status and monthly distribution of typhoid in Bushenyi.

Results: Among the males surveyed between sentinel centres, age group 10–19 years had the highest typhoid prevalence of 28.6% (Comboni) and 36.6% (BMC), while age group 20–29 years had 24.6% (IAH) highest typhoid prevalence. Among the females surveyed age group 10–19 years had the highest typhoid prevalence of 33.8% (Comboni) and 32.8% (BMC), while age group 20–29 years had 24.3% (IAH) highest typhoid prevalence. Typhoid was more prevalent in the low class group and lowest in the high class group across the sentinel centres. It appears that typhoid prevalence is uniformly distributed from January to December.

Conclusions: Typhoid is highly prevalent among febrile patients attending clinics in Bushenyi. Typhoid prevalence was significantly (p < 0.05 & p < 0.01) dependent on age and sex, not socio-economic status and season. Hygiene education and monitoring of the street-food trade is recommended typhoid control measure.

**P966** Validation of a new stratification score to predict infection due to antibiotic-resistant bacteria

M. Salvadó*, E. Calbo, M. Riera, M. Salamero, N. Freixas, M. Xercavins, M. Rodriguez-Carballeira, J. Garau (Terrassa, ES)

Introduction: Relation with health care system has been associated with a higher risk for resistant pathogens (RP) and a greater mortality than community acquired infections; however not all component criteria for HCAP convey a similar risk for resistance. Recently, a new score to determine which patients with pneumonia were more likely to be infected by RP has been published.

Objective: To validate this score system in a cohort of patients with health care associated bacteraemia (HCAB).

Material and Methods: From Jan 2006 to Oct 2008, consecutive adult patients with HCAB were identified through the records of the Clinical Microbiology Laboratory in a 500-bed acute care hospital. Definition of HCAB included: residence in a nursing home in the previous month, hospitalisation in an acute care hospital for 48h or longer in the 90 days before, haemodialysis treatment 30 days before admission or receive IV therapy, wound care, enteral nutrition, indwelling urinary catheter manipulation or health care at home in the 30 days before the HCAB. This scoring system used assesses 4, 3, 2 and 1 points to recent hospitalisation, nursing home residence, haemodialysis and intensive care unit (ICU) admission, respectively. Patients with a higher score were more likely to have resistant pathogens. RP included MRSA, Extended Spectrum β-Lactamases (ESBL) producing Enterobacteriaceae, *P. aeruginosa* and other non fermenting Gram negative rods, as described previously in the referred score.

Results: Among a cohort of 321 HCAB patients, RP were recovered in 42 (13%) [8 MRSA, 11 ESBL, 22 *P. aeruginosa* and 1 Acinetobacter]. The prevalence of RP ranged from 10% to 13% and was not significantly different in those with previous hospitalisation, on haemodialysis or ICU admission; patients coming from a nursing home were more likely to have RP (21% vs. 10%, p = 0.007). Among patients with fewer than 3 points the prevalence of RP was 10%, 11.5% in those with a score ranging from 3 to 5 and more than 37.5% in those >6 (p < 0.001). The sensitivity and specificity of a score >6 was 21.4% and 95%, respectively.
ESBLs, AmpCs & others in Enterobacteriaceae: genes, plasmids & clones – part 1

Conclusions: Residence in a nursing home is associated with HCAAB due to resistant pathogens. The sensitivity of the new scoring system used is very low in areas where the prevalence of RP is low and is not a good tool to predict infection by RP in these areas.

ESBLs, AmpCs & others in Enterobacteriaceae: genes, plasmids & clones – part 1

Spanish nationwide study on Klebsiella pneumoniae producing extended-spectrum β-lactamases (GEIH-BLEE 2006). Emergence of CTX-M-15

C. Ruiz de Alegría*, M.E. Cano, M.A. Díaz, E. Román, J.R. Hernández-Bello, J. Rodríguez-Baño, A. Pascual, L. Martínez-Martínez and GEIH

Objectives: To study the susceptibility testing and types of extended-spectrum β-lactamases (ESBL) in clinical isolates of K. pneumoniae collected in a nationwide study performed in Spain in 2006.

Methods: Consecutive K. pneumoniae with an ESBL-production phenotype obtained from diagnostic clinical samples (1 per patient) in 44 centres representing all regions in Spain were included. Identification was confirmed by API 20E. Susceptibility to β-lactams and confirmation of ESBL production were performed with ESBL-Plus panels (Dade, Microscan), and susceptibility to other agents by standardised microdilution (CLSI guidelines). Resistance was defined with EUCAST breakpoints. Clonal relationship was performed by Rep-PCR and, for some isolates, with pulsed-field gel electrophoresis. Beta-lactamase genes were characterised by PCR in parental strains (representative of clonal groups and resistance phenotypes) and in ESBL-producing transconjugants selected with E. coli J53 Azide-Resistant and cefotaxime (2mg/l) or ampicillin (100mg/l). Specific primers for TEM, SHV and CTX-M were used; when CTX-M genes were detected a second PCR for specific groups was performed. ESBL-encoding genes were identified by sequencing.

Results: One hundred and sixty-four isolates (78 clones) were isolated from 31 centres (1 to 16 isolates and 1 to 9 clones per centre). Percentages of resistance (all isolates) were: 9% (imipenem, meropenem), 2% (ampicillin), 5% (ertapenem), 9% (ticarcillin), 50% (Piperacillin/Tazobactam, gentamicin), 62% (ciprofloxacin), 66% (cotrimoxazole), 68% (tobramycin) and 96% (Amoxicillin/clavulanate). In 114 parental strains evaluated, amplicons for CTX-M, SHV and TEM were obtained in 80 (70%), 107 (94%) and 55 (48%) isolates. SHV and TEM genes were only sequenced in transconjugants. ESBL identified in 78 transconjugants included CTX-M (71%), SHV (26%) and TEM (6%). CTX-M-15 and SHV-12 were identified in 18 and 14 of 31 centres, respectively, and both enzymes in 6 centres.

Conclusion: K. pneumoniae producing ESBL are widespread in Spain. CTX-M-15 and to a much lesser extent SHV-12 were the more common enzymes found in this study.

Diversity of extended-spectrum β-lactamases in Escherichia coli: second nationwide study in Spain (GEIH-BLEE 2006)


Objectives: To describe the diversity of extended-spectrum β-lactamases (ESBL) types and susceptibility pattern in clinical isolates of E. coli obtained in a nationwide study performed in Spain (2006).

Methods: Forty-four hospitals representing all regions of Spain participated in the study. All consecutive clinical isolates (1/patient) with a phenotype compatible with ESBL production from February-March 2006 were included. Identification was confirmed by API system (bioMérieux). ESBL production was confirmed by microdilution using ESBL-Plus panels (Dade, Microscan). In a selection of strains, susceptibility to antimicrobials and ESBL confirmation was performed by microdilution (or diffusion) according to CLSI guidelines. ESBL encoding genes were characterised by PCR. Final identification of ESBL-encoding genes was performed by sequencing.

Results: The total number of ESBL-producing E. coli isolated were 1,021; 257 isolates were selected and used for further analysis. Two hundred and sixty-four ESBLs were identified among the 257 E. coli selected strains, distributed as follows: CTX-M (73%), SHV (26%), and TEM (1%). Sequence analysis of 249 ESBL selected genes in E. coli yielded: CTX-M-14 (116 isolates), CTX-M-28 (43), CTX-M-9 (21), CTX-M-32 (5), CTX-M-27 (1), CTX-M-1 (2), CTX-M-22 (1), CTX-M-79 (1), SHV-12 (56), TEM-52 (2), and TEM-4 (1). The most active antimicrobial agents were tigecycline and carbapenems (100% of isolates were susceptible), followed by amikacin (98.1%), fosfomycin (93.7%), piperacillin/tazobactam (87%), gentamicin (78.4%) and amoxicillin/clavulanate (66.9%). Only 30.1% of strains were susceptible to ciprofloxacin.

Conclusions: There was a great diversity in ESBL types among clinical isolates of E. coli in Spain in 2006. CTX-M being the most prevalent family and CTX-M-14 the most frequent enzyme.
This clonal group belongs to phylogenetic B2 group and it shares several plasmidic multiresistant determinants as aac(6′)-Ib-cr and blaOXA-1. The detection of an emerging clone is the first step to the design of prevention and possible intervention strategies. The aim of this study is to characterise the CTX-M-15-producing strains among ESBL-producers E. coli isolated in our area.

**Methods:** Two collections of clinical ESBL-producing E. coli isolates were analyzed. 54 were obtained between June 2005-August 2006 and 80 between September 2006-February 2007; and 67 strains isolated from raw poultry meat were also included. Identification and antimicrobial susceptibility were performed by standard methods. ESBL production was tested by the double-disk method and characterisation was carried out by PCR with specific primers for bla-genes and sequencing. Presence of aac(6′)-Ib-cr and blaOXA-1 were screened by PCR and sequencing. Isolates were serotyped, compared by XbaI PFGE and phylogenotypes were assigned by multiplex PCR.

**Results:** A total of 21 CTX-M-15 isolates were detected. An increase of clinical CTX-M-15-producing strains was observed from 4% in the first period to 24% in the second period and they were obtained from urine (71%), blood (24%) and pleural effusion (5%). Two (3%) food ESBL-producers were positive for this enzyme. All the isolates from urine (71%), blood (24%) and pleural effusion (5%). Two (3%) food ESBL-producers were positive for this enzyme.

**Conclusions:** The increase of CTX-M-15-producing E. coli isolates observed recently in our area is mainly due to clone O25b:H4-ST131 (B2 phylogenetic group). One cluster (>85% similarity) was identified by PFGE containing 11 isolates O25b:H4-ST131 which was positive for aac(6′)-Ib-cr and blaOXA-1. For six of strains belonged to phylogenetic group A were included in the same cluster by PFGE (2 clinical isolates and 2 food isolates).

**Objective:** To analyse an outbreak of broad-spectrum cephalosporin-resistant K. oxytoca strains in a paediatric intensive care unit to determine their resistance genes

**Methods:** Eleven K. oxytoca isolates were recovered from clinical specimens (haemoculture, bronchial aspirate, conjunctive secretions) from 5 patients during a 5-month period (2006–2007). Antibiotic susceptibility patterns were determined using Microscan® system and by agar dilution method. The screening of ESBL production was checked by the double disk test (CLSI). Isolates were typed by PFGE with XbaI. The presence of β-lactamase genes, qnr, qepA, aac(6′)-Ib-cr, aac(3)-II and tet genes and their genetic environments were analysed by PCR and sequencing. Amino acid changes in GyrA and ParC proteins were studied by PCR and sequencing.

**Results:** All K. oxytoca isolates presented a multiresistance phenotype that included resistance to ciprofloxacin, trimethoprim, sulfamethoxazole, gentamicin, tobramycin, and tetracycline, in addition to β-lactams, and ESBL-production was identified in all cases. The MIC of cefotaxime, cefazidime, nalidixic acid, ciprofloxacin, levofloxacin, gentamicin, amikacin and tobramycin were of 264, 32, 8–16, 4–8, 2, 128, 8, and 64 mg/L, respectively. All isolates showed an indistinguishable or closely related pattern by PFGE. The gene encoding CTX-M-15 was found in all isolates, being this gene surrounded by IS2627 and orf7777. All isolates harboured blaOXA-1, qnrS1, aac(3)-II and tet(A) genes. In addition, they contained two copies of aac(6′)-Ib-cr gene, one of them included in the aac(6′)-Ib-cr-IS26-aac(6′)-Ib-cr-blaOXA-1 structure, and the other one in the aac(6′)-Ib-cr-IS26-mntI structure, being this last one non previously reported. The M157L amino acid change was identified in ParC protein in all isolates, although the wild sequence was obtained for GyrA. All patients survived and the outbreak was finally controlled after special control measures.

**Conclusion:** The current reporting rate is the importance of blaCTX-M-15-containing K. oxytoca as nosocomial pathogens in intensive care unit outbreaks, and the coexistence with genes associated with quinolone (aac(6′)-Ib-cr, and qnrS1) or aminoglycoside resistance (aac(3)-II, aac(6′)-Ib-cr). Two copies of aac(6′)-Ib-cr genes were identified in our strains, one of them with a new genetic environment.

**Objective:** Shifts in ESBL-epidemiology might impose new therapeutic and infection control approaches, justifying their periodic surveillance. In a previous survey (2003–05) performed in a tertiary care Portuguese hospital, Hospital S. Teotónio, a low incidence of ESBLs (only TEM variants -10, -24 and -116) mainly associated with E. aerogenes epidemic clones was described. In this work we report the recent changes in ESBL-types occurred in this institution.

**Methods:** A total of 1146 Enterobacteriaceae were isolated during one year period (2006–07). Species identification and susceptibility testing were performed by standard methods. ESBL characterisation included synergy test and identification of known bla genes (blaTEM/SHV/CTX-M) by PCR and sequencing. Presence of the recently described qepA gene was also searched by PCR.

**Results:** ESBL production was observed in 5% (81/1486) of the isolates. ESBL-producers were identified as E. coli (n = 48), E. cloacae (n = 14), K. pneumoniae (n = 10), K. oxytoca (n = 4), M. morganii (n = 1), S. marcescens (n = 1), and P. mirabilis (n = 1). Co-resistance to non-β-lactams was frequently observed, mainly to tetracyclines (78%), kanamycin (78%), tobramycin (70%), gentamicin (70%), streptomycin (60%), ciprofloxacin (60%), and sulfonamides (58%). qepA genes were absent. Nine ESBL-types were observed, being identified as TEM (-10, -52, -57, -116) (19/81, 23%), SHV (-12, -64) (18/81, 22%) or CTX-M (-14, -15, -32) (38/81, 47%). The most common ESBL types were CTX-M-15 (35/81, 43%), recovered from E. coli and SHV-12 (18/81, 22%) or CTX-M (-14, -15, -32) (38/81, 47%). The most common ESBL types were CTX-M-15 (35/81, 43%), recovered from E. coli and SHV-12 (18/81, 22%) or CTX-M (-14, -15, -32) (38/81, 47%). The most common ESBL types were CTX-M-15 (35/81, 43%), recovered from E. coli and SHV-12 (18/81, 22%) or CTX-M (-14, -15, -32) (38/81, 47%).

**Conclusion:** A rapid increase and diversification of ESBL-types and -producing species was observed in this hospital, being especially prevalent CTX-M-15 and SHV-12 producers. Dissemination of epidemic clones and plasmids carrying these ESBLs also conferring resistance to other antibiotics seems to have contributed to this shift.

**Objective:** ESBLs have become widespread in hospitals and in communities. The CTX-M genotypes in particular have spread and diversified rapidly. Over 50 CTX-M-β-lactamases are recognised, which divide into five clusters, CTX-M-1, 2, 8, 9, 25 based on sequence homology. In the UK, over 90% of ESBLs have been shown to belong to group 1 CTX-M, with CTX-M15 predominating.

The aim of this study was to investigate the molecular epidemiology and prevalence of CTX-Ms in Enterobacteriaceae collected in the Royal Free Hospital and adjacent surgeries in north London.

**Methods:** Clinical isolates of Enterobacteriaceae were collected from June until August, 2008. CTX-M genes were detected by PCR using two sets of primers to differentiate CTX-M1, 3, 10, 11, 12, 15, 55,
23, 28, 29, 30 ("M3 like") from "M14 like" (M9, 13, 14, 16, 17, 18, 19, 21, 24, 27) (Chia JH et al 2005 J Clin Micro 43: 4486–91). Amplicons of 479 base pairs indicated the presence of CTX-M-like alleles and 355 bp indicated CTX-M-like alleles. Amplicons were then sequenced with another set of primers, CTX-F and CTX-R1, which corresponded to nucleotide positions −19 to +2 and +894 to +877 on CTX-M3 plasmid, respectively (Chia, JH et al, 2005 J. Clin. Micro. 43:4486–91). Data analysis was performed using Bionumerics® software and results were compared using the BLAST nucleotide database and Clustal W2 alignment programme for DNA.

**Results:** Twenty nine isolates were CTX-M producers, with CTX-M15 the most prevalent (Table 1). CTX-M15/28 varies from CTX-M15 by only two nucleotide positions and has no impact on resistance phenotype.

**Conclusions:** Antibiotic sensitivity and ESBL detection methodologies currently in use do not distinguish the ESBL genotype. The genotype has implications for susceptibility to other antibiotics, such as gentamicin and piperacillin-tazobactam. The ability to rapidly type ESBLs could also inform infection control practices, particularly when investigating an outbreak. Adaptation of this rapid molecular method to routine clinical diagnostics is planned. Rapid and accurate screening would greatly benefit patients as it would optimise the effectiveness and timeliness of treatment. Optimal antibiotic treatment would also reduce the further development of antibiotic resistance.

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of strains (%)</th>
<th>CTX-M-vce</th>
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<tbody>
<tr>
<td>E. coli</td>
<td>124 (62.0)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>29 (14.5)</td>
<td>1 (3.45)</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>10 (5.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Others</td>
<td>37 (18.5)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>5 (2.5)</td>
</tr>
</tbody>
</table>

**Table 1. Prevalence of CTX-M expressing ESBL organisms**

ESBLs, AmpCs & others in Enterobacteriaceae: genes, plasmids & clones – part 1

255

Y. Glupczynski

Objectives: As part of national two-yearly surveillance, we assessed the species distribution and characterisation of the different types of ESBLs among broad-spectrum cephalosporin-resistant Enterobacteriaceae isolates prospectively collected in Belgian hospitals during the first trimester of 2008.

**Methods:** Maximum 10 consecutive, unduplicated clinical Enterobacteriaceae isolates resistant to 3rd and/or 4th generation cephalosporins were collected in each participating centre and sent to a reference laboratory for central testing. All strains were confirmed as ESBLE by double combination disk test (DDT) and/or by ESBL E-tests (ceftazidime and ceftriaxone + clavulanic acid). ESBLs were characterised by isoelectric focusing and PCR-sequencing assay targeting blaTEM, blaSHV, blaCTX-M and blaOXA. The genetic environment of ESBL genes was analysed by PCR mapping and DNA sequencing. Phylogenetic group were assigned by multiplex PCR targeting chuA, yjaA, svg genes and the TSPE4C2 element. Clonality was assessed by PFGE.

**Results:** ESBL-EC isolates harboured TEM alone (n = 68), CTX-M-TEM (n = 49), CTX-M alone (n = 10), SHV alone (n = 3), CTX-M-SHV (n = 1), TEM-SHV (n = 1). DNA sequencing of CTX-M-EC revealed CTX-M1 isolates in 44 isolates (including 33 CTX-M-15, 10 CTX-M-1 and 1 CTX-M-2) and CTX-M-2 or CTX-M-9 group in 8 and 5 isolates. Among CTX-M-producing E. coli, 82% harboured TEM-1 and 57% OXA-30 enzymes, whereas co-production of SHV was rare (2%). CTX-M producing strains belonged to phylotypes B2 (67%), A (21%), D (7%) and B1 (5%). PFGE showed 25 genotypes in CTX-M-producing E. coli. Majority (27/33) of CTX-M-15 isolates belonged to a major PFGE type found in 18 centres (1 to 4 isolates/centre) suggesting clonal spread of CTX-M-15 in Belgium. In the remaining isolates, CTX-M-15 could not be found upstream to CTX-M-3. 65% transposition element was only detected upstream to CTX-M-2.

**Conclusion:** This study showed that CTX-M producing E. coli strains are widely distributed in Belgian hospitals. Molecular analysis indicates this derives from both gene dissemination (CTX-M-2 and CTX-M-9) and epidemic spread of CTX-M-15 producing clone in approximately half of these centres. Further study is in progress to establish the relationship of this epidemic strain to the international O25:H4-ST131 clone.

**[P974] Epidemiology of ESBL-producing Enterobacteriaceae in Belgian hospitals: results of nationwide study in 2008**


Objectives: As part of national surveillance project, we analysed the diversity of extended spectrum β-lactamases (ESBL), resistance genes, genetic environment, clonality and phylogenetic background of ESBL-producing E. coli (ESBLEC) isolated in Belgian hospitals.

Methods: A total of 132 unduplicated clinical ESBLEC isolates collected between 01/2006 and 06/2006 in 40 (maximum of 5 strains per centre) were sent to a reference laboratory. The presence of ESBL was confirmed by double combination disk test (DDT) and/or by ESBL E-tests (ceftazidime and ceftriaxone + clavulanic acid). ESBLs were characterised by isoelectric focusing and PCR-sequencing assay targeting blaTEM, blaSHV, blaCTX-M and blaOXA. The genetic environment of ESBL genes was analysed by PCR mapping and DNA sequencing. Phylogenetic group were assigned by multiplex PCR targeting chuA, yjaA, svg genes and the TSPE4C2 element. Clonality was assessed by PFGE.

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Phylogenetic background of CTX-M-EC strains over a period of 8 years in a University hospital in Belgium.

**Methods:** In 2000–2007, E. coli clinical isolates were screened for ESBL production and confirmed by double-disk synergy test and/or combined-disks. ESBL producing E. coli isolates (n=288) from blood (n=23), urine (n=117) and rectal swabs (n=148) were included in this study. ESBLs were characterised by multiplex PCR for bla genes of the SHV, TEM and CTX-M family, CTX-M group determination and DNA sequencing. Phylotyping was performed by multiplex PCR targeting chuA, yjaA, svg genes and the TSPE4C2 element. PCR. ESBL-EC (n=112) strains were tested for MIC of 12 antimicrobials by agar dilution. The genetic environment of CTX-M-1 group was analysed by PCR mapping and DNA sequencing.

**Results:** ESBL-EC strains harboured CTX-M (57%) TEM (37%) or SHV enzymes 10%. The proportion of CTX-M enzymes increased from 25% in 2000 to 69% in 2004 and levelled off in the last 4 years. Among CTX-M-EC, CTX-M group 1 was predominant followed by group 2 (75 and 16% of CTX-M enzymes, respectively). The proportion of group 1 increased (30% in 2000 to 90% in 2001). CTX-M group 1 was flanked by a truncate ISEcp1 and orf477 transposition elements in 91% of isolates. CTX-M producing strains belonged to phylogenetic groups B2 (59%), A (22%), B1 (10%) and D (9%). The proportion of phylogroup B2 in rectal swabs isolates was similar to those from blood or urine. In ESBL-EC, meropenem, amikacin and temocillin were the most active antibiotics in vitro with MIC90 of 0.06 mg/mL, 8 mg/mL and 16 mg/mL, respectively. Co-resistance to ciprofloxacin, cotonaxazole and tobramycin was 54, 58 and 48% respectively.

**Conclusions:** In our hospital, CTX-M enzymes were the most frequent ESBL among EC in recent years. CTX-M of group 1 was predominant (75%) and appeared located within the same element of transposition as CTX-M-15. The majority of CTX-M producing isolates belong to virulent phylogroup B2. Contrary to previous reports this phylogroup was as frequent in rectal isolates as in urine or blood isolates. Those data document an important reservoir of ESBL genes among virulent E. coli strains at this hospital.

**Conclusion:** ESBL-EC have clearly emerged in our hospital (6.3% of all E. cloacae isolates compared to 3.7% and 2.6% during the same period one and two years before). Clinical epidemiological data, phenotypic identification to subspecies and molecular typing all suggested a polyclonal dissemination with possible horizontal gene transfer originating from strains that were predominantly recovered from patients hospitalised in ICUs. Continuous active surveillance is warranted in microbiology laboratories for the early detection of ESBLs and the prevention of outbreak in infrequent ESBL-producing species such as E. cloacae.

**Methods:** In this study, ESBLs were characterised by multiplex PCR for bla genes of the SHV, TEM and CTX-M family, CTX-M group determination and DNA sequencing. Phylotyping was performed by multiplex PCR targeting chuA, yjaA, svg genes and the TSPE4C2 element. PCR. ESBL-EC (n=112) strains were tested for MIC of 12 antimicrobials by agar dilution. The genetic environment of CTX-M-1 group was analysed by PCR mapping and DNA sequencing. Phylotyping was performed by multiplex PCR targeting chuA, yjaA, svg genes and the TSPE4C2 element. PCR.

**Results:** In 2000–2007, 86, TEM-130, TEM-132, SHV-1 and SHV-5 enzymes were detected. The proportion of SHV enzymes 10%. The proportion of CTX-M enzymes increased from 25% in 2000 to 69% in 2004 and levelled off in the last 4 years. Among CTX-M-EC, CTX-M group 1 was predominant followed by group 2 (75 and 16% of CTX-M enzymes, respectively). The proportion of group 1 increased (30% in 2000 to 90% in 2001). CTX-M group 1 was flanked by a truncate ISEcp1 and orf477 transposition elements in 91% of isolates. CTX-M producing strains belonged to phylogenetic groups B2 (59%), A (22%), B1 (10%) and D (9%). The proportion of phylogroup B2 in rectal swabs isolates was similar to those from blood or urine. In ESBL-EC, meropenem, amikacin and temocillin were the most active antibiotics in vitro with MIC90 of 0.06 mg/mL, 8 mg/mL and 16 mg/mL, respectively. Co-resistance to ciprofloxacin, cotonaxazole and tobramycin was 54, 58 and 48% respectively.

**Conclusions:** In our hospital, CTX-M enzymes were the most frequent ESBL among EC in recent years. CTX-M of group 1 was predominant (75%) and appeared located within the same element of transposition as CTX-M-15. The majority of CTX-M producing isolates belong to virulent phylogroup B2. Contrary to previous reports this phylogroup was as frequent in rectal isolates as in urine or blood isolates. Those data document an important reservoir of ESBL genes among virulent E. coli strains at this hospital.
Results: phenotypic analysis revealed the presence of ESBLs in 32 isolates of E. coli (25.8%), 63 isolates of K. pneumoniae (73.2%) and 5 isolates of K. oxytoca (45.5%). E. coli isolates were found to carry SHV-ESBL (N=1), CTX-M-1 (N=3), CTX-M-3 (N=4), CTX-M-15 (N=13), CTX-M-14 (N=3) and a novel variant of CTX-M-2 designated CTX-M-2a (Ala208Thr) (N=6). K. pneumoniae isolates were found to carry SHV-ESBL (N=25), CTX-M-3 (N=5), CTX-M-15 (N=21), CTX-M-2 (N=5), CTX-M-2a (N=13). K. oxytoca – CTX-M-15 (N=2), CTX-M-2 (N=1), CTX-M-2a (N=1). Two K. pneumoniae isolates coexpressed SHV-ESBL together with CTX-M-15 and one isolate together with CTX-M-3. All E. coli and majority of Klebsiella producing CTX-M-15 were found to carry OXA-1 type β-lactamase. All E. coli and Klebsiella isolates producing CTX-M-2 and CTX-M-2a were OXA-2 type β-lactamase positive. The majority of Klebsiella and E. coli ESBL producers were multidrug resistant expressing resistance to two or more non-β-lactam antibiotics. The common resistance profile for K. pneumoniae CTX-M-15 producers was CIP GENTOBNIT (N=16) and for E. coli CTX-M-15 producers GAT CIP TOB NIT (N=13) with additional GEN resistance being expressed in 11, SXT – in 8 isolates. Klebsiella spp and E. coli isolates expressing CTX-M-2, CTX-M-2a, CTX-M-3 and SHV-ESBL were mainly GEN TOB resistant. CTX-M-14 producing E. coli isolates were not resistant to non-β-lactam antibiotics GAT CIP TOB AMI NIT SXT.


Objective: To investigate the molecular epidemiology and genetic features of extended-spectrum β-lactamase (ESBL) producing K. pneumoniae epidemic clone (KP-EC) with unusual resistance pattern isolated from multiple nosocomial outbreaks and sporadic cases between 2006 and 2008 in Hungary.

Methods: As result of continuous monitoring of ESBL-producing KP-ECs 28 isolates collected from 5 healthcare facilities submitted to the National Center for Epidemiology were selected for macrorestriction analysis by PFGE. Of these 12 strains were isolated from adult inpatients (including 5 invasive samples from one nosocomial outbreak) in 2 healthcare facilities and 16 strains were isolated from newborns (including 12 invasive samples from two nosocomial outbreaks) in 4 healthcare facilities. The MIC values were determined by agar dilution technique for the following antibiotics: ceftazidime, cefotaxime, gentamicin, amikacin and ciprofloxacin. Furthermore molecular typing was performed by PCR and sequencing of several antibiotic resistance genes, plasmid profile analysis, transfer of resistance determinants and multilocus sequence typing (MLST).

Results: All isolates showed moderate resistance to ciprofloxacin (MICs ranged from 0.5 mg/L to 8 mg/L). The MICs for ceftazidime proved 64–128 mg/L in the “adult isolates” and 8 mg/L in the “newborn isolates”. PFGE revealed the existence of only one genetic cluster defined as EC IV. PstI digestion of plasmid DNA from transconjugants/transformants revealed two highly diverse restriction patterns corresponding to “adult” and “newborn isolates”. Sequence analysis of β-lactamase genes from plasmids of 15 selected isolates detected blaSHV-2a in strains isolated exclusively from newborns and blaCTX-M-15 in strains isolated exclusively from adult inpatients. MLST established that strains of the PFGE cluster belonged to a novel sequence type ST 274. ESBL-producing K. pneumoniae isolates belonging to the novel sequence type ST 274 adapted to the newborn and adult hospital settings in Hungary by acquiring SHV-2a or CTX-M-15 type enzymes, respectively. Thus, new strategy for exceptional adaptation to different hospital settings was found in KP population.

Objective: To characterize extended-spectrum β-lactamase (ESBL) genes present in chromosomal AmpC-producing entero bacteriaceae isolates recovered in a Bulgarian cancer hospital.

Methods: Screening for genes encoding ESBLs (blaPER-1, blaTEM, blaCMY, blaCTX-M, blaVEB) was carried out by PCR amplification with specific primers in 37 non-duplicate, clinically relevant ESBL-producing isolates including 19 Citrobacter freundii, 9 Enterobacter cloacae, 5 Serratia marcescens, 2 Enterobacter aerogenes, 1 Morganella morgani and 1 Providencia rettgeri. For isolates with PCR-positive results, sequencing was performed. Susceptibility to antimicrobials was determined by standard disk diffusion or Etest procedures.

Results: The 37 chromosomal AmpC-producing Enterobacteriaceae were found to coproduce the following ESBLs: 14 (37.8%) CTX-M-3, nine (24.3%) TEM-3, eight (21.6%) SHV-2, four (10.8%) CTX-M-15, one (2.7%) TEM-15 and one (2.7%) PER-1. Sixteen isolates (43.2%) also carried blaTEM-1, and one of them carried blaSHV-1 as well. In vitro, all isolates were susceptible to imipenem. Susceptibility to other drugs was as follows: 78% for ciprofloxacin, 43% for amikacin and 32% for gentamicin. Associated resistance to amikacin and ciprofloxacin was observed most frequently among CTX-M-positive isolates.

Conclusions: The most prevalent ESBLs were CTX-M enzymes (CTX-M-3 and CTX-M-15) followed by TEM-3 and SHV-2. This is the first report of TEM-15 and PER-1-producing Enterobacteriaceae in Bulgaria.
**Antimicrobial susceptibility testing of extended-spectrum β-lactamase producing Enterobacteriaceae isolated from Macau, China**

T. Ling*, J. Lei, C.C. Lee, K.T. Wong, K.V. Koon (Hong Kong, HK; Macau, MO)

**Objective:** ESBLs emerged in the 1980s and now have been reported throughout the world. The consequences of ESBL-mediated resistance in the clinical setting can be tremendous and lethal. This is the first study that we present antibiotic susceptibility profile, prevalence rate and genotypes of ESBL producers in Macau.

**Materials and Methods:** The MICs of amikacin (AMK), ciprofloxacin (CFX), piperacillin-tazobactam (PTZ) and imipenem (IMP) were determined using GNS-121 and 137 cards loaded to the VITEK system following the CLSI recommendations. ESBL production was determined by a 5 mm increase in zone diameter for either cefotaxime or cefotaxime in combination with clavulenate versus its zone size when tested alone. The ESBL enzymes were characterised by multiplex PCR according to the method of Colom which detects and discriminates between blaSHV, blaTEM and blaOXA-1 PCR amplicons of 392, 516 and 619 bp respectively. CTX-M type ESBL enzymes were characterised by multiplex PCR according to the method of Xu which detects and discriminates between CTX-M-gp 1, CTX-M-gp 2, CTX-M-gp 8, 25/26, 619 bp respectively. CTX-M type ESBL enzymes were characterised in combination with clavulenate versus its zones size when tested alone.

**Results:** A total of 697 clinical Enterobacteriaceae isolates were collected during Oct 2007 to Jul 2008 in CHCSJ and the results showed that 26.9% of E. coli and 21.6% of Klebsiella spp. were ESBL producers. The resistance rate of ESBL producing organisms to AMK, PTZ and CFX were 3.8%, 8.2% and 71.4% respectively. All strains were sensitive to IMP. Among 150 ESBL producing E. coli, 59.3% were producing TEM-type, 6.7% OXA-type; 4% TEM+OXA-type and 30.0% did not produce TEM, SHV or OXA-type enzymes. Among 30 ESBL Klebsiella spp., 26.7% SHV-type, 16.7% TEM-type and 1 OXA-type. 30% SHV+OXA-type, 2 produced TEM-SHV-type and 5 did not produce TEM, SHV or OXA-type enzyme. In this study, E. coli (90%) and Klebsiella spp. (80.0%) were producing CTX-M-type ESBL. Among E. coli, 59.3% CTX-M-gp 9 type, 28.0% CTX-M-gp1 type and 4 both CTX-M-gp 9 type and CTX-M-gp1 type. Among Klebsiella spp., 63.3% CTX-M-gp 9 type, 16.7% CTX-M-gp1 type ESBL.

**Conclusion:** ESBL producing rate of E. coli and Klebsiella spp. were 26.9% and 21.6% respectively. All ESBL producers remain sensitive to IMP; however, there was a high resistance rate of more than 70% to CFX. CTX-M is the predominant type of enzymes found in both ESBL producing E. coli and Klebsiella spp.

**Molecular characterisation and epidemiology of Enterobacteriaceae isolates other than Escherichia coli and Klebsiella spp. that are non-susceptible to extended-spectrum cephalosporins in Thailand**

P. Kiritisarn*, A. Hemprasert (Bangkok, TH)

**Objectives:** To (1) characterise β-lactam resistant genes, (2) survey the susceptibilities to antimicrobial agents and (3) demonstrate molecular epidemiology of extended-spectrum cephalosporin-resistant Enterobacteriaceae other than Escherichia coli and Klebsiella spp. (EOTEK) in Thailand.

**Methods:** Non-duplicate clinical isolates of EOTEK were collected at Siriraj Hospital, a 2200-bed university hospital in Bangkok (Thailand) during October 2006-March 2007. Antimicrobial susceptibilities were tested by disc diffusion and E-test® methods. Isolates resistant to an extended-spectrum cephalosporin were tested for ESBL production based on the CLSI phenotypic method, and were detected for ESBL genes by PCR. AmpC gene detection was performed for isolates that were present with ESBL genes but negative for ESBL test. All PCR products were sequenced to identify their molecular types. Pulsed-field gel electrophoresis (PFGE) analysis was used to demonstrate their genetic relationships.

**Results:** A total of 143 out of 598 isolates (23.9%) of EOTEK were not susceptible to extended-spectrum cephalosporin(s), including Enterobacter (n=91), Salmonella (n=20), Proteus (n=11), Citrobacter (n=9), Serratia (n=6), Providencia (n=4) and Morganella (n=2). ESBL genes were detected in 142 isolates (99.3%) and included CTX-M-15 (53.5%), TEM-116 (43.7%), CTX-M-55 (16.9%), VEB-1 (16.2%), CTX-M-3 (14.8%), SHV-12 (13.4%), CTX-M-40 (2.1%) and SHV-2a (0.7%). Only 100 isolates (70.4%) of ESBL gene-carrying isolates were positive for ESBL test. Among 42 ESBL phenotype-negative isolates that harboured ESBL genes, 34 isolates (81%) also possessed AmpC gene(s). The MIC90 (ug/mL) of ESBL gene-carrying isolates against cefotaxime, ceftriaxone, cefazidime, ciprofloxacin, imipenem, meropenem, ertapenem and doripenem were >256, >256, >256, >32, 1.5, 0.5, 6 and 0.19, respectively. 20.4% of isolates were shown to be non-susceptible to ertapenem. PFGE analysis demonstrated that multiple clones were present.

**Conclusion:** We found that ESBL genes, particularly CTX-M-15, were very common among cephalosporin-resistant EOTEK. However, the phenotypic detection of ESBL suggested by the CLSI was not efficient, probably due to the high prevalence of AmpC production among these isolates. We first report the detection of TEM-116 in Thailand. Susceptibility to ertapenem was noticeably reduced as compared to other carbapenems. Based on the PFGE analysis, most of these isolates had no clonal relationship.

**Rampant resistance to β-lactam antibiotics in previously sus-ceptible Enterobacteriaceae: the Kwazulu-Natal experience**

E. Essack*, U. Gocinden, C. Mocktar, U. Ramgulam (Durban, ZA)

**Objectives:** Molecular mechanisms of resistance were investigated in Escherichia coli, Salmonella spp. and Proteus mirabilis, enterobacteriaceae previously fully susceptible to all β-lactam antibiotics ranging from penicillins to carbapenems.

**Methods:** E. coli and P. mirabilis were obtained from a multi-centre surveillance study instituted in 16 hospitals at 3 progressive levels of health care (district, regional, and tertiary) where microbiology laboratories collected 100 consecutive, non-replicative isolates. Salmonella spp. were obtained from a collection of putative extended-spectrum β-lactamase-producers from a tertiary hospital. Representatives of particular resistance phenotypes determined by susceptibility testing using the CLSI Kirby Bauer disc diffusion method were subjected to isoelectric focusing, plasmid profile analyses and detection of β-lactamase genes by PCR and sequencing.

**Results:** Sequencing evidenced TEM-145 and TEM-146, new inhibitor-resistant β-lactamase genes and CMY-20, a new plasmid-mediated AmpC-type β-lactamase gene, in addition to the OXA-1, TEM-55, SHV-2, CTX-M1 and TEM-1 genes in the 38 E. coli isolates. TEM-1, SHV-1 and TEM-53 were found in the 29 P. mirabilis isolates while 41 Salmonella spp. expressed one/more of the SHV-2 SHV-12, TEM-63, TEM-116, TEM-131, CTX-M-3, CTX-M-15, CMY-2 and OXA-1 β-lactamase genes. Diverse β-lactamase genes and/or enzyme combinations and plasmid profiles indicated extensive mobilisation of resistance genes.

**Conclusions:** The complex and diverse patterns of β-lactamase genes suggest an epidemiology where β-lactamase production has become endemic, and where evolution is generating a wide range of enzyme combinations complicating patient management and rendering useless a once highly efficacious antibiotic armamentarium in a public healthcare system dependent on syndromic management and empirical therapy.
The world among us: tropical and parasitic diseases

Medical intervention at immigrant camps in Greece
O. Adamis*, A. Spilioti, I. Keramidas, T. Sideroglou, T. Papadimitriou, I. Pierrousatskos (Athens, GR)

Objectives: Registration and evaluation of typical medical screening of migrating populations, with emphasis on epidemic infectious diseases, at camps where illegally arrived immigrants, at first stay.

Methods: The staff of the Office for Mobile Populations of the HCDCP conducts medical intervention regarding health inspection of illegally arrived persons in Greece, in specific places where these persons are kept temporarily after they are arrested. The typical screening of the intervention involves physical examination, obtaining the immigrant’s medical and social history. Information is registered in the database of the HCDCP. Data analysis is based on descriptive statistics.

Results: The screening conducted on 4505 persons (4020 male, 485 female) during the period of 09/01/2007–22/12/2007. Mean age was 24.21 years (± 8). In reference with the declared countries of origin, 1123 (24.92%) immigrants were from Afghanistan, 1180 (26.26%) from Iraq, 338 (7.50%) from Pakistan, 626 (13.9%) persons were from Palestine, 706 (15.67%) from Somalia and 532 (11.80%) from other countries (India, Bangladesh etc.). Health problems were detected at 1029 (22.84%) cases. 365 (8.1%) cases of dermatological diseases, 310 (6.89%) cases of respiratory diseases, 120 (2.66%) cases of gastrointestinal system diseases, 77 (1.71%) cases of myoskeletal system diseases, 61 (1.35%) cases of urinary system diseases, 23 (0.51%) cases of cardiovascular system diseases and 73 (1.62) cases of other systems were registered. 53 (1.18%) persons were referred for further clinical assessment by specialists, 40 persons (0.89%) for laboratory tests and treatment was given to 438 of them (9.72%).

Conclusions: In case of mass arrival of immigrants, the aim is the safe treatment of them with respect to international humanitarian principles. Main priority is the immediate intervention, so the state mechanism for immediate interference is activated, if cases of emergence infectious diseases are detected.

Protocol for congenital Chagas disease control in Florence, Italy

Objectives: Chagas disease causes high morbidity in many Latin American countries. Maternal-fetal transmission of Trypanosoma cruzi occurs in 2–12% of pregnant infected mothers. Early treatment of infected infants attains an early 100% eradication rate. In August 2008, the Tuscany Reference Centre for Tropical Diseases implemented a protocol for the screening of congenital Chagas disease at the main public maternity hospital (Careggi Hospital) of Florence, Italy.

Methods: The programme consists of: 1) screening of pregnant women coming from endemic areas for seroreactivity to T. cruzi, by using an immunochromatographic assay (ICT) Chagas Quick Test, Cypress Diagnostics, Belgium) and conventional T. cruzi IgG ELISA test (CHAGAS IgG ELISA, Nova Tec, Germany), 2) serological (ICT plus ELISA) and parasitological (microscopic examination and nested polymerase chain reaction-PCR with primers TCZ1/TCZ2 and TCZ3/TCZ4, followed by sequencing) evaluation of infants born to T. cruzi-infected women, 3) treatment of infected infants. Infants are considered infected in case of microscopic detection of T. cruzi, or PCR positivity in at least two different samples, or seropositivity at 8 months of age.

Results: From 1/8 to 31/12/2008, 35 pregnant women were screened (mean age 31 years, limits 14–40). The countries of origin were: Peru (17), Brazil (6), Argentina (3), Bolivia (2), Colombia (2), El Salvador (2), Chile (1), Costa Rica (1) and Venezuela (1). All except one mother tested negative. The seroreactive mother, aged 29 years, came from Bolivia. The further evaluation of her infant is ongoing. The parasitological evaluation at 1 month of age was negative.

Conclusions: In Europe, the presence of immigrants from endemic areas makes possible the appearance of congenital infection in newborns of mothers living with chronic Chagas disease. To increase the early detection of congenitally infected infants and thus facilitate their early treatment, surveillance of pregnant women from endemic areas is recommended. Furthermore, the detection of a maternal Chagas infection should lead to extend the screening to other family members.

Travel-associated enteric fever: a review of demographics, clinical features, laboratory findings, treatment and outcomes in a UK district general hospital
V. Sreeharan*, M. Laundy (Oxford, Slough, UK)

Objectives: To identify the demographics, clinical and laboratory features of enteric fever in a UK hospital over a 5 year period, and to review the management and outcomes of these cases.

Methods: This is a retrospective study of 41 blood culture confirmed cases of Salmonella typhi and Salmonella paratyphi between 2003 and 2008 with available medical notes. Patient demographics, clinical features, treatment and outcomes were obtained from the notes, and laboratory data from the hospital database.

Results: Demographics: Ages ranged from 3 years to 66 years (median 21 years), male:female (60%:40%), and all had a history of recent travel (55% India, 30% Pakistan, 5% Sri Lanka). Clinical features: Onset of symptoms occurred whilst abroad in 37% and on return to the UK in 63% (median duration on arrival was 10 days). Symptoms included fever (100% with 78% rigor), gastrointestinal (90% with 68% diarrhoea, 46% abdominal pain, 7% constipation), non-specific features (93% including malaise, anorexia, fatigue, myalgia, headache, dizziness) and cough (24%). Signs included fever (93%), gastrointestinal (51% with 14% abdominal tenderness, 15% hepatomegaly and 10% splenomegaly), bradycardia (5%) and rose spots (2%). Laboratory findings: Raised C-reactive protein (100%) and raised alanine transaminase (88%) were the most consistent findings. The blood culture organism was S typhi (39%), S paratyphi A (59%) and S paratyphi B (2%), with the proportion of S paratyphi A cases increasing over the study period. 54% were ciprofloxacin resistant with the proportion of resistant cases increasing over time. Treatment and outcomes: 93% received appropriate antibiotics, with no known negative outcomes for those who did not. There was 1 severe case of enteric colitis, 1 case of known relapse and no known mortality. Time to defervescence was variable (1–16 days, median 4 days) and length of stay varied from 1–18 days (median 6 days).

Conclusion: A diagnosis of enteric fever must always be considered in patients with fever and a recent travel history. Clinicians in areas with a high proportion of patients from South Asia should be particularly familiar with the diagnostic features and the high levels of ciprofloxacin resistance.

Effective tick removal with a fishing line knot
G. Ghirga*, P. Ghirga (Cistudeucechia, IT)

Objective: The Centers for Disease Control recommends tick removal using a pair of fine-tipped tweezers to firmly grasp the tick very close to the skin and with a steady motion, pulling the tick’s body away. Unfortunately, this method carries a high risk of crushing tick’s body (thorax, head) particularly for tiny species. This may force infective body fluids through the tick’s mouthparts into the wound site. We report the results of an open study aimed to investigate if ticks attached to human skin can be effectively removed by tightening a knot of a fishing line around the tick’s mouthparts.

Methods: Ten ambulatory children between the age of 4 and 13 entered the study. Seventeen ticks attached to children’s skin were removed by two physicians, a paediatrician and a dermatologist, tightening a knot of a fishing line around the tick’s mouthparts. Technique: Use a 20 cm
fishing line, diameter 0.4 mm or thinner. Slowly tie a simple knot around the tick’s head. If the line is pressed against the skin while being gently pulled, the knot will tighten around the tick’s mouthparts. Pull the ends of the line slowly and steadily until the tick can be eased out of the skin. Avoid tightening the knot sharply, as this may tear the mouthparts from the body of the tick, leaving them embedded into the skin.

**Results:** All ticks detached as the knot was tightened. Once the ticks were removed they were placed in 70% ethanol for microscopic evaluation of the mouthparts. The ticks were all less than 0.5 mm. Twelve ticks were completely removed. The remaining have the mouthparts almost completely removed. All ticks were alive. On follow up no patient showed sign of local infection or rickettsiosis.

**Conclusions:** Tick removal by tightening a knot of a fishing line around the tick’s mouthparts appears to have a high success rate however areas covered with hairs may make this method difficult to apply. It seems particularly indicated for removal of tiny species.

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**Phylogenomic and subproteomic approaches to gain new insights into the mitochondrion features of the Cryptosporidium parvum parasite**

L. Patignan*, A. Petrucco, P. Jedelsky, F. Del Chierico, C. Russo, L. Mancinelli, J. Wastling, J. Tschezy, D. Menichella (Rome, Chieti, IT; Prague, CZ; Liverpool, UK)

**Objectives:** Cryptosporidium spp. is a protozoan parasite that causes widespread diarrheal disease in humans and other animals and is responsible for large waterborne outbreaks of cryptosporidiosis. Unlike many organisms belonging to the phylum Apicomplexa, such as Plasmodium spp. and Toxoplasma gondii, there is no clinically proven drug treatment against this parasite. In our study an analysis of the mitochondrial proteome of C. parvum, performed by exploiting genomic and proteomic approaches, has allowed us to infer preliminary metabolic maps and hypothesize evolutionary implications.

**Methods:** Genomic entries from C. parvum genome project (Abrahamsen et al., Science 304 5669: 441–5) were analysed for EC and GO classification, leader peptide signals and transmembrane regions and matched against CryptoDB annotations (http://cryptodb.org/cryptodb/) to provide an updated list of mitochondrial ORF and peptides. Organellar proteomics was performed on: i) mitochondrial fractions, from oocysts/sporozoites, by using 1D-PAGE LC/MS-MS; ii) mitochondrial and mitochondrial/reticulum endoplasmaticum fractions, from purified sporozoites (DEAE cellulose/pH gradient), by using iTRAQ quantitative labelling and RP-HPLC/MS-MS. Phylogenomics was performed by using ClustalW, Phylip 3.6 and MEGA 3 softwares.

**Results:** Genomic and subproteome analyses have identified mitochondrial related peptides from which metabolic pathways associated to a modified oxidative phosphorylation system (OXPHOS) have been inferred. In particular, the mitochondrial NADH dehydrogenase and the alternative oxidase associated to the OXPHOS, the pyridine nucleotide transhydrogenase related to the inner membrane electron transport, the superoxide dismutase involved in the reactive oxygen species (ROS) scavenging system, and proteins implicated in the importing processes have been characterised. Phylogenomics analyses of the loci have provided inference for evolutionary strategies developed by C. parvum to adapt its mitochondrial metabolism to low oxygen conditions typical of the host gastrointestinal tract.

**Conclusion:** Our data aim to provide new insights into the metabolism of C. parvum, describing peculiar biochemical pathway of the proteome network, suggesting new ideas for the adaptation evolutionary strategies of the parasite and new potential drug targets. However, to gain further advances into the metabolism of C. parvum we need to persist with the development of more precise sub-proteome studies.

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**Alpha-tocopherol transfer protein gene disruption confers resistance to protozoan infections**

M. Herbas*, X. Xuan, H. Arai, H. Suzuki (Obihiro, JP)

**Objective:** The nutritional status of the host influences susceptibility or resistance against protozoan infection. Micronutrient deficiency, such as vitamin E might play a protective role against infection. Mice deficient in alpha-tocopherol transfer protein (alpha-TTP), with undetectable levels of vitamin E in circulation, were used as a model to analyze the effect of vitamin E deficiency on the outcome of protozoan infections, in order to propose a new strategy for the prevention and control of protozoan infections by modification of the nutritional status of the host.

**Methods:** alpha-TTP knockout mice were infected with Plasmodium berghei or Trypanosoma congolense at lethal doses, and their survival rates and parasitemia were recorded. DNA damage of parasites was evaluated by means of comet assay and anti-8-OHdG test and antioxidant defence system in parasites was examined by monitoring the mRNA expression of antioxidative stress enzymes by real time quantitative PCR.

**Results:** alpha-TTP knockout mice infected with P berghei or T. congolense survived significantly longer than the wild type mice (p < 0.05). The percentage of parasitemia in alpha-TTP knockout mice infected with P berghei, remained at very low levels during the acute phase of infection. Whereas, parasite density in the knockout mice infected with T. congolense remained at very low levels compared to wild type mice (p < 0.01). Comet assay revealed clear comet tails in the parasites infecting alpha-TTP knockout mice, such tails were not observed in parasites infecting the wild type mice. In addition, anti-8-OHdG test revealed a positive reaction in the parasites infecting the alpha-TTP knockout mice. Furthermore, mRNA expression of antioxidative stress enzymes from parasites infecting alpha-TTP knockout mice were significantly up-regulated after infection (p < 0.05). Expression levels were significantly higher in parasites infecting knockout mice as compared to parasites infecting wild type mice (p < 0.05).

**Conclusion:** Inhibition of alpha-TTP confers resistance to the development of protozoan infections, and this resistance is induced by oxidative damage of the parasites due to vitamin E deficiency in the circulation of the host. Inhibition of alpha-TTP activity might be an interesting alternative for the control, prevention and treatment of protozoan infections.

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**Iron supplementation for children in malaria-endemic areas: systematic review and meta-analysis**

J.U. Ojukwu, J. Okebe, D. Yahav, M. Pial* on behalf of the Cochrane Infectious Diseases Group

**Objectives:** Iron supplementation has been claimed to increase the risk for malaria among children living in malaria-endemic areas. We assessed the effect of iron on malaria and malaria-related adverse events, including deaths.

**Methods:** Cochrane systematic review and meta-analysis of individually and cluster randomised-controlled trials conducted in hyperendemic to holoendemic malaria regions. Trials comparing orally administered iron/ -folie-acid vs. placebo or no treatment for children <18 years old were included. Iron fortification was excluded. Antimalarials and/or antiparasitics could be administered to either group. Additional micronutrients could be administered only equally to both groups. The
primary outcomes were malaria-related events and deaths. Secondary outcomes included haemoglobin, anaemia, other infections, growth, hospitalisations and clinic visits. Pooled relative risks or absolute mean differences are presented with 95% confidence intervals. Adjusted analyses were obtained or computed for cluster-randomised trials.

**Results:** Sixty-four trials compared iron vs. placebo for prevention or treatment of anaemia. Iron supplementation did not increase the risk for clinical malaria 1.03 [0.96–1.12], 13 trials, 21,105 children. The risk was similar among non-anaemic children. The risk for malaria parasitaemia was higher with iron, 1.13 [1.01–1.26], but there was no difference in adequately concealed trials. Iron supplementation increased haemoglobin by about 1 g/dL in malaria hyperendemic settings, with significant heterogeneity. Lower haemoglobin at baseline was associated with significantly higher effects and co-supplementation with zinc or antiparasitics with smaller effects. Malaria endemicity did not affect results. Other secondary outcomes were not affected, but for increased risk for diarrhoea with zinc co-supplementation and fewer clinic visits with iron. Iron with antimalarial vs. placebo (4 trials) decreased malaria, hospital admissions and anaemia. Iron vs. placebo given for treatment of malaria (3 trials) did not increase risk for parasitological failure (0.98 [0.69, 1.39]). There was no increased risk for death across all trials comparing iron vs. placebo, 1.06 [0.69–1.62], 12 trials, 20,712 children.

**Conclusions:** Considering the overall evidence, iron does not increase the risk for clinical malaria or death. Recommendations regarding iron supplementation for children living in malaria-endemic areas should consider these results.

**[P993] Uncovering the secrets of East African relapsing fever**
S. Cutler* (London, UK)

**Objectives:** To gain further insights into the relationship between the louse-borne *Borrelia recurrentis* and its tick-borne counterpart, *Borrelia duttonii*. Relapsing fever, caused by spirochaetes belonging to the genus *Borrelia*, were once a worldwide epidemic disease. Despite a global reduction, disease remains a significant burden in several African nations. Indeed, tick-borne relapsing fever (TBRF) is usually listed within the top 10 causes of mortality in children under five in Tanzania. In Ethiopia, louse-borne relapsing fever (LBF) is also within the top 10 causes of hospital admission, associated with significant morbidity and mortality.

**Methods:** The recent publication of full genome sequences for *B. recurrentis* and *B. duttonii* relapsing fever spirochaetes has provided opportunity to gain insights into the relationship of these organisms, with *B. recurrentis* appearing as a louse-borne derivative of the tick-borne *B. duttonii*. Furthermore, several intriguing features were noted for *B. recurrentis* such as the apparent truncation of recA and smf genes together with a frame shift in mutH, possibly hastening the evolution of this spirochaete. We explored whether these observations were a feature of more than the single isolate for which the genome has been sequenced and whether this could have been introduced through selective pressures of in vitro cultivation. Both questions were assessed using analysis of spirochaetal DNA directly in patient serum samples, thus avoiding selective pressures through in vitro cultivation. Furthermore, we have explored the phylogenetic relationship among these spirochaetes from both Tanzania and Ethiopia through sequencing an intragenic spacer region (IGS).

**Results:** The clustering of IGS types previously observed for these species using cultivated spirochaetes and those detected among arthropod vectors was confirmed using 123 sera from patients form Ethiopia and Tanzania. Similarly, the geographical demarcation of these spirochaetes was called into question, with the finding of IGS types resembling *B. crocidurae* normally found in Western Africa. Interestingly, the novel spirochaete described in tick samples was not detected in any human samples calling into question its human pathogenic potential.

**Conclusions:** We were able to confirm the apparent damage to DNA repair genes directly in situ within LBF patient clinical material. The apparent phylogenetic overlap between LBF and TBF spirochaetes was also confirmed.

**[P994] Entamoeba histolytica or dispars through PCR and Elisa II?**
Y.A. Öner*, E. Okaygün (Istanbul, TR)

**Objectives:** Amoebic infections are serious parasite diseases that cause invasion and damage in organs such as liver, lungs and brain besides intestinal colonisation. For many years diagnosis of amebiasis was confirmed by microscopic identification of *E. histolytica* cysts and trophozoites in stool. But then, it was realised that there are nonpathogenic Entamoeba species (*E. dispers*) in a similar morphological structure with *E. histolytica*, so ELISA and PCR are used for diagnosis more often nowadays. In our study, we used a multiplex PCR method to distinguish *E. histolytica* and *E. dispers* and compare the results with the ones of ELISA and microscopy.

**Methods:** Eighty-three stool samples sent to the Microbiology and Clinical Microbiology Department which were determined to have Entamoeba cysts were taken into our study group. Adhesion was examined in our study group through Techlab E. Histolytica II ELISA kit. Parasite DNA was investigated through multiplex PCR method, using primers specific to *E. histolytica* (EhP1 and EhP2) and *E. dispers* (EdP1 and EdP2).

**Results:** 40 out of 83 microscopy-positive stool samples were positive after applying the Techlab E. histolytica II ELISA kit and E. histolytica DNA was positive in 48 samples through PCR-method.

**Conclusion:** It was impossible to distinguish the pathogen (*E. histolytica*) and non-pathogen (*E. dispers*) Entamoeba species through direct stool microscopy. PCR-method has not been in common practice due to its high cost and the requirement of experienced staff. ELISA II kit determining *E. histolytica* adhesin should be preferred due to its high sensitivity-specificity and low cost and ease of use.

**[P995] Real-time characterisation of cytoadhesion of Plasmodium falciparum by using a novel biosensor technology (thickness shear mode)**

**Objective:** A medically relevant phenomenon of malaria tropica (*P. falciparum*) is that late so-called signet-ring-stages cannot be detected in the peripheral blood. These stages adhere to endothelial cells of postcapillary venules by receptor-ligand binding and thereby escape from clearance in the spleen. In this work it is planned to investigate to which point of time infected erythrocytes, expressing *Plasmodium falciparum* erythrocyte membrane protein 1 ( PfEMP1) on their surface, bind to ligands of endothelial cells. Measurement will be done first-time by Thickness Shear Mode (= TSM).

**Methods:** The innovative TSM-method is based on the principle that binding of particles or cells onto the surface of the quartz alters its resonant frequency. In relation to malaria research we are able to realise experiments with human whole blood over a period of 48 hours in the platform. This is an essential preconditon for experiments with infected erythrocytes. Aim of this project is the optimisation and development of a novel sensitive measurement of the binding behaviour of signet-ring-stages to endothelial cells. Previous methods depend on a pure optic registration of bound erythrocytes in cell culture with the disadvantage that these methods are not accurate enough for a clear definition of binding times. Furthermore they are very time-expensive. By analysing and interpreting the signals obtained by TSM, information on mass and physical behaviour of the attached objects can be gained. This means that quick, reproducible and automated tests can be run which is a significant advantage over conventional methods.

**Results:** At present, immobilisation of the ligands CD36 and CSA is finished. Coating of the quartz's surface with these ligands was proven by both immunofluorescence and TSM measurements. Furthermore we could detect adherence of late trophozoites and schizonts to both ligands by use of TSM. Adequate controls with uninfected red blood cells have been carried out parallel where no binding effect could be demonstrated.
Conclusions: The innovative TSM-method can give new insights into the adherence procedure of P. falciparum, so this could provide possible points of attack for the use of drugs against this dangerous infectious disease.

![Coagulation abnormalities in imported malaria-clinical and laboratory findings](image)

J. Nikolic, G. Stevanovic, M. Pelemis, M. Paunovic *(Belgrade, RS)

Objectives: Evaluation of coagulation profile, through assessment of clinical and laboratory indicators, in patients with imported malaria. The association with disease severity and prognosis was determined through comparison with parasitaemia, disease severity and manifest bleeding.

Methods: A retrospective case-control study included 81 travelers from endemic regions, diagnosed and treated for malaria in Institute for Infectious and Tropical diseases, Belgrade, Serbia, from January 1998 to January 2008.

Results: Age, sex, use of prophylaxis, previous immunity and species of Plasmodium, did not have a significant effect on coagulation profile (p > 0.05). Only two patients (2.5%) had bleeding manifestations-pauchial haemorrhage and splenic infarction, but there was no evidence of disseminated intravascular coagulation (DIC). Laboratory findings included significant thrombocytopenia, prolonged prothrombin time and activated thromboplastin time with D-dimer elevation (p < 0.05). There were no significant changes in antithrombin III levels or fibrinogen (p > 0.05). Severe malaria was associated with thrombocytopenia, which was reversible after 5 days of antimalarial therapy. There was a positive correlation between parasitaemia and levels of D-dimer and antithrombin III, while thrombocyte count was in negative correlation with parasitaemia (p < 0.05).

Conclusion: Although hypercoagulability is often observed in malaria, bleeding manifestations and need for supportive therapy are uncommon.

![Awareness, possession and use of insecticide-treated net for prevention of malaria in children under five in Abeokuta, Nigeria](image)


A survey was carried out to assess awareness, possession and use of Insecticide Treated Net (ITN) by mothers in preventing malaria among children under-five. Malaria though was considered dangerous by almost all respondents (98.5%); the level of awareness of ITN as a malaria preventive tool was 75.1% while possession was 45%. Awareness and possession of ITN were positively and significantly influenced by high educational qualification of mothers and attendance of a public hospital for antenatal care. Hospitals were identified as the major source of awareness among respondents; Women that delivered their babies in Traditional birth home displayed least awareness (38.6%) and recorded low possession (10%). There was no significant relationship between ITN usage, birth order and age of child. Heat experienced while sleeping under ITN and problem of how to hang the net were major limitations identified in the use of ITN. The need to involve women receiving antenatal care outside the hospital in malaria control intervention is hereby recommended.
Survey through questionnaires in Madagascar: preliminary study on possible risk-cofactors during childhood in the transmission route of human herpes virus-8 with promoter blood sucking arthropods

R. Romano*, L. Mannocci, F. Tabacchi, G.M. Paganotti, M. Coalse (Rome, IT)

Objectives: The Human herpesvirus-8 (HHV-8) infection occurs mainly in Africa with an high level in paediatric age and this suggest the existence of an alternative way of transmission beside the sexual intercourse. The presence of HHV-8 in saliva of seropositive subjects led to consider this fluid as the source of infection. Our study is based on the hypothesis of “promoter arthropod”, according to this the promoter arthropods play a role in the transmission of virus when mother’s saliva is used to relieve itching and scratching on the bite site of children. Our aim, as in other surveys in sub-Saharan African countries is to observe the presence of possible risk-cofactors related to the virus infection route such as: (i) use of traditional methods based on saliva and premasticated herbs used on children’s skin to relieve itching, (ii) the local inflammatory reaction related to child’s hypersensitivity response to the bite and (iii) systemic symptoms such as fever.

Methods: We carried out survey with questionnaires directed at 224 children (age 5−13) interviewed at schools in four villages located in a south-eastern area of Madagascar.

Results: Questionnaires revealed the following frequencies: use of traditional methods 3.6% (respectively saliva 2.2% and herbs 1.3%), fever after bite 54.9%, skin irritation 63.8%, swelling 32.1% and skin mark 64.7%, lasting of skin boring: <3 days 46.4%, 3−7 days 15.2% and >7 days 6.2%. The association between traditional methods and fever is slightly significant (p < 0.038), the variable fever shows statistically significant direct relationship with the presence of skin mark (p < 0.004) and with the lasting of skin boring time >7 days (p < 0.001).

Conclusion: Analysis of data shows that skin reaction and fever are possible risk-cofactors in the transmission route of HHV-8. Further surveys on the presence of HHV-8 DNA virus in mother’s saliva will be important to confirm our hypothesis that blood sucking promoter arthropods are involved in the transmission of virus. This could be an attempt to control HHV-8 transmission addressed to a cohort of HHV-8 seropositive mothers to make them aware on the risks of the use of saliva to relieve insect bites, and on the far safer habit to protect children against arthropod bites with insecticide treated nets (ITNs), repellents or anti-histamnic products to relieve the itching.

Entomological survey of plebotomine sand flies in a focus of visceral leishmaniasis in a regional centre of Portugal (Coimbra municipality)

M. Pereira*, O. Afonso, M. Machado, M.C. Sousa (Coimbra, Lisbon, PT)

Leishmaniasis, zoonoses caused by protozoans of the genus Leishmania, has been the object of considerable attention of both human and veterinary medicine. Leishmania parasites are transmitted by female sand flies of the genus Phlebotomus in the Old Word and Lutzomyia in the New Word. P. ariasi and P. perniciosus species are the biologic vectors of leishmaniasis canine in the Mediterranean basin include Portugal. An entomological survey of plebotomine sand flies was conducted in a focus area of canine leishmaniasis, in Coimbra municipality of Portugal. Standardised sampling with Centers for Disease Control (CDC) light traps was employed to determined monthly trends is species composition, density and sex ratio. A total of 992 sandflies (569 males and 353 females) were collected from June 2008 to October 2008. Four species representing two genera were identified: three Phlebotomus species (P. perniciosus, P. ariasi and P. sergentii) and one Sergentomyia species. Phlebotomus perniciosus was the predominant species, comprised 69% of the sand fly population, followed by P. ariasi (23.4%), S. minuta (7.3%) and P. sergentii (0.3%). A population peak (August) was observed for P. perniciosus, suggesting a uni-modal annual pattern. Considering the high density of P. perniciosus and P. ariasi, the area of Coimbra should be considered as a potential focus of L. infantum. Abundance was greatest in non urban areas and in the yard (68% of the phlebotomines captured), in the vicinity of households. This is the first description of plebotomine sand flies species in the municipality of Coimbra, in region centre of Portugal.

Brucella melitensis endocarditis accompanied with Candida albicans in a patient with prosthetic valve


Objectives: Although the incidence of Brucella and Candida endocarditis is low, their courses are associated with high mortality rates. We report a patient with prosthetic valve who had Brucella melitensis endocarditis accompanied with Candida albicans, successfully treated with medical and surgical intervention.

Case report: A 27-year-old male was admitted with a history of fatigue, loss of appetite and fever. He had mechanical aortic and mitral valve replacement fifteen years ago. He had a history of consumption of unpasteurised dairy products.

Physical examination revealed a temperature of 37.5°C, hepatosplenomegaly and in cardiac examination a grade 5/6 aortic diastolic murmur was detected. In laboratory examination: WBC: 9100/mm³, haemoglobin: 7.4 g/dl, ESR: 84 mm/h, CRP: 56.4 mg/dl and serum Brucella agglutination titre was 1/80.

Transthoracic echocardiography demonstrated minimal mitral and tricuspid regurgitation in addition to moderate aortic regurgitation. Two vegetations with 1×0.4 cm and 1×0.5 cm diameters were detected on the ventricular side of aortic valve leaflet.

Vancomycin plus gentamicin therapy was initiated empirically. At day 5th, Brucella melitensis was isolated from the cultures of blood. The antibiotic treatment was switched to rifampicin (600 mg/d p.o.), doxycycline (100 mg p.o., bid) and trimethoprim-sulfamethoxazole (TMP-SMZ; 160 mg TMP and 800 mg SMZ p.o., bid). At day 14th, the patient required mechanical ventilation support due to respiratory failure. At day 30th, the patient still had mechanical ventilation support and fever plus leucocytosis developed. From the repeated blood cultures Candida albicans was isolated. Caspofungin was added to the treatment. At day 43th, he underwent aortic valve replacement because of unresponsiveness to medical treatment, aortic valve dysfunction and left ventricular failure. Cultures of the excised aortic valve yielded C. albicans.

During postoperative course no surgical complication occurred and the rifampicin, doxycycline, TMP-SMZ and caspofungin treatment was administered until discharge. The patient recovered fully without sequelae and discharged at day 94th. The triple antibiotic regimen for brucellosis was continued for 3 months after the discharge.

Conclusion: In prosthetic valve endocarditis due to Brucella melitensis and Candida albicans, surgical replacement of the vegetated valve, and postoperative treatment with appropriate antibiotics, is the best options for curing this disease.

Trypanosoma cruzi infection diagnosis in a Spanish health department during 2008

M.J. Gimenez*, E. Calabuig, A. Gil, M.D. Gomez, J. Lopez Aldueger, M. Gobernado (Valencia, ES)

Objective: Chagas’ disease is a vector-borne disease endemic in Latin America. In Europe, where the triatomine vector is not present, transmission of Trypanosoma cruzi infection can be either mother-to-child or through organ transplant or blood transfusion; early diagnosis and treatment could avoid it. Since January 2008, T. cruzi infection screening must be done to all Latin American pregnant women by order in council at the Valencian region (Spain).

The aim of this study was to describe the main features of the newly diagnosed patients with Chagas’ disease in our Health Department during 2008.
Visceral leishmaniasis in Serbia – treatment problems

G. Stecanovic*, M. Pelemis, Z. Dukic, M. Puclovic, L. Lacadinovic, J. Polaga (Belgrade, RS)

Visceral leishmaniasis is parasitic diseases caused by Leishmania donovani (L. infantum, L. chagasi). Reservoirs of the parasites in Serbia region are mostly dogs and rodents. Vector of transmission is sand flies. Illness was sporadically occurred in the southern region of Serbia.

Methods: During 2001–2008 periods in our department we treated 25 patients safer from visceral leishmaniasis. Diagnosis was established by serological methods (Leishmania dipstick rapid test Diasys Europe), and definitive diagnosis was done by microscopic examination of bone marrow smears.

Results: In endemic regions of Serbia and Montenegro, 18 patients were lived, others were been during summer period in these regions. No one was traveled out of Europe. All the patients were adults, average age of 40.24 (range from 22–78) years, 17 of them was males and 8 were females. Medium duration of the illness before treatment was longer then 4 mounts. Most of them had fever, anaemia or pancytopenia and enlargement of liver and spleen.

As a primary therapy we used antimony (Glukantime®) in the doses of 20 mg/kg during 21–28 days in 23 of our patients. In one patient we used Pentostam®.

In two patients we used liposomal amphotericin B (Ambisome®), as a first choice therapy. And they were cured.

Good outcome we have in 17 patients, initial treated with antimony. But in 5 patients in spite of therapy clinical, findings were present. Splenomegaly and pancytopenia was persisted, with pancytopenia.

In patients with persistent findings of parasites we repeated therapy with antimony compounds. One of patient had good outcome, but other 4 needed amphotericin B. All of them were treated during 15–28 days, given intravenously for a total dose of 20 mg/kg. After two courses of amphotericin B therapy, only two patients had persisted clinical findings longer then 6 months. These two patients were treated with liposomal amphotericin B (Ambisome®) in daily dose of 2 mg/kg during 5 days. Resolution of the symptoms was achieved during first month after the therapy.

Conclusion: Unresponsiveness to antimony therapy is becoming problem in Asia. In former Yugoslavia, we did not have such problems, few years ago. Now it became increasing problem and amphotericin B of different formulation will became a drug for primary therapy. Other drugs such as miltefosine were not present on Serbian market. Favourite outcome was achieved by use of liposomal amphotericin B.
Evolutive pattern of trichinellosis in patients of a Roma community

I. Marincu*, L. Negrutiu, I. Iacobiciu, A.M. Neghina, C. Oancea, R. Neghina (Timisoara, RO)

Objectives: Trichinellosis remains a major public health issue in Romania because of diagnostic difficulties and multiple complications. The present study aims to conduct a retrospective investigation of the evolutive and clinical pattern of trichinellosis in a group of patients belonging to the roman community.

Methods: The authors have retrospectively analyzed the hospital records of 88 Rromi patients (48 males, 40 females) inhabitants of 3 endemic areas for trichinellosis in Timis county, Romania, and hospitalised in the Clinic of Infectious Diseases from Timisoara during the period 2002–2007. The positive diagnosis was based on epidemiological elements (the onset of infection following consumption of pork infested with *Trichinella spiralis*), clinical elements (fever, headache, repeated chills, nausea, diarrhoea, facial or periorbital edema, myalgia) and laboratory tests (erythrocyte sedimentation rate, fibrinogen, IgM Ab anti-Trichinella, leucocyte value, eosinophil value, serum protein electrophoresis). The statistical processing of data was done using the Epi Info 5 program.

Results: Of the study group 31 patients (35.27%) were inhabitants of 2 rural areas (26 patients from Ieaca Mare, 5 from Padureni) and 57 patients (64.77%) lived in urban areas (Timisoara). The clinico-biological pattern presented as follows: 86 patients (97.72%) had dispeptic digestive syndrome, 70 patients (79.54%) had fever over 38°C, 55 patients (62.50%) presented periorbital edema, 22 patients (25.00%) had non-specific eruptions, 18 patients (20.45%) had pulmonary involvement, and 5 patients (5.68%) had myocarditis. According to the severity of the disease 23 patients (26.13%) presented asymptomatic clinical forms, 25 patients (28.40%) had mild forms, 35 patients (39.77%) had moderately severe forms and 5 patients (5.68%) had severe clinical forms. The source of trichinellosis was the consumption of infested pork, without prior veterinary inspection. Winter was the season with most cases (70.45%). All patients had a favourable outcome following treatment with albendazole (Zentel) or mebendazole (Vermox) for 7−10 days.

Conclusions: Trichinellosis is a widely spread zoonosis among the Rromi belonging to the roman community. In Romania because of diagnostic difficulties and multiple complications. The present study aims to conduct a retrospective investigation of the evolutive and clinical pattern of trichinellosis in a group of patients belonging to the roman community.

Methods: In January 2008, an outbreak of trichinellosis due to consumption of infected pork involved 15 people in localities Carpini and Peciu Nou. The epidemiological investigation performed by the Department of Public Health in Timis County and the medical records of the patients hospitalised at Victor Babes Hospital of Infectious Disease in Timisoara were the main sources for data collection. Identification of *Trichinella* sp larvae at species level was performed by Multiplex PCR at Istituto Superiore di Sanita, Rome, Italy (CRLP code 223/08) following the isolation of the larvae from an infected meat sample by artificial digestion.

Results: All 15 cases consumed pork from the same backyard pig which was slaughtered in a household without veterinary inspection of the meat. The infective species was *Trichinella spiralis*. Five patients presented clinical forms of the disease and were hospitalised. The following considerations include only the hospitalised cases. All cases were serologically confirmed (IgG antibodies against Trichinella in a positive titer). The mean age of the trichinellosis patients was 33.4 years (range 22–53). The symptomatology occurred 17 days following the consumption of the infected meat and all patients presented headache, eyelid and lower limb oedema, myalgia, fever, diarrhoea and nausea. The eosinophil values ranged between 5.5% and 52%. The outcome of the hospitalised patients was favourable under treatment with Albendazole, Dexamethasone and Calcium lactate. The nonhospitalised patients consulted an infectiologist and were ambulatory treated with Albendazole for 7 days.

Conclusion: Even if the current European regulations aim at ensuring that only meat that has been certified trichinella-free after systematic control may be consumed, the people who slaughter backyard pigs are not always aware about this issue. Trichinellosis outbreaks, as an important public health concern, reinforce the need to urgently implement veterinary and educational programs. Identification of *Trichinella spiralis* species is in accordance with the findings of other Romanian studies and strengthens the supposition that it is the most frequently spread of the species in our country.

**Expression of K2S form of human tissue plasminogen activator in trypanosomatid protozoa Leishmania tarentolae**

R. Nazari*, N. Daudiri (Qom, Tehran, IR)

Objective: Tissue plasminogen activator protein (t-PA) is one of the most important thrombolytic agents for treating of cardiovascular obstructions such as stroke and efforts is currently focused to improve the t-PA molecule and thereby its pharmacokinetic properties. K2S is a derivative t-PA that has a longer half-life and greater resistance to inhibitor than the natural t-PA molecule. The Leishmania tarentolae expression system represent the combination of easy handling known from bacterial expression systems with the potential of an eukaryotic protein expression/folding/modification system. The aim of this research is cloning and expression of K2S form of the t-PA cDNA in eukaryotic system L. tarentolae.

Methods: cDNA of t-PA was made by RT-PCR from human blood cells. PCR with specific primers for producing truncated form of t-PA (K2S) were used. For introducing the K2S form of t-PA in Leishmania tarentolae cells, we constructed plasmid pFXmlsp1.4hkg-K2S. After development of construct, electroporation was done on L. tarentolae cells for transfecting developed construct. Western blot analysis and zymography analysis for evaluation of expression and biological activity of recombinant K2S was performed.

Results: The construct (pFXmlsp1.4hkg-K2S) were confirmed by restriction analysis and PCR. After electroporation and screening of cells, diagnostic PCR analysis showed integration of K2S expression cassette in 18 SSr mRNA (18 s) gene. We replaced the native signal sequence of t-PA gene with a signal sequence derived from the secreted acid phosphatase of *L. mexicana*. This study revealed that using Leishmania derived signal sequence yielded secretion of recombinant K2S form from transformed L. tarentolae. Then production of recombinant K2S was confirmed by Western blot analysis on supernatant of transformed L. tarentolae culture. Performance of zymography analysis
on supernatant of transformed *Leishmania* culture showed clear zone as a result of K2S function. The test confirmed serine protease activity and natural biological activity of recombinant K2S. Thus we showed heterologous proteins as complex as K2S produced in an active form in leishmania tarentolae.

**Infections in animal models**

**P1011 Identification of pertinent indicators of virulence in a mouse model of Acinetobacter baumannii pneumonia**

M. Excillard*, M. Kempf, C. Solter, M.L. Joly-Guillou (Angers, FR)

**Objectives:** Animal models have been developed to study *Acinetobacter baumannii* (Ab) pathogenesis which is still poorly understood. Our objective was to identify patient indicators of virulence in a mouse model of Ab pneumonia.

**Methods:** Five bacterial strains isolated from patients with nosocomial infections (AB-M, SAN, AYE, AB-NM, CIP 53.77) were used. These strains were inoculated by intra-tracheal instillation of 5x10⁶ CFU in C3H/HeN mice rendered transiently neutropenic. Each strain was inoculated in 40 mice separated in 2 groups; one group to assess spontaneous outcome and another group to assess virulence according to the weight and a clinical score (CS) built on the basis of mice mobility, the development of conjunctivitis, and the aspect of hair. Mice were followed during 7 days. Concurrently, bacterial counts in lungs were performed. Inflammatory response was assessed by the dosage of MIP-2 and TNF-α in lungs. To identify pertinent indicators of virulence, correlations with mortality were studied for bacterial counts, CS, maximal loss of weight (MLOW), and concentrations of pro-inflammatory mediators. Correlations were studied by Spearman correlation coefficient (r) analysis and the Fisher F-test.

**Results:** Strain SAN was highly virulent (78.9% mortality), strain AB-M intermediately virulent (48.4% mortality), and the 3 others were more weakly virulent (24.0%, 18.8% and 13.0% for strains AYE, AB-NM and CIP 53.77 respectively). Bacterial counts in lungs, clinical score, MLOW and concentrations of pro-inflammatory mediators varied according to strains. There was a significant correlation between our CS and mortality (r = 0.85; P < 0.05), whereas there was not any between the MLOW and mortality (r = 0.33), and between the bacterial counts in lungs and mortality (r = 0.56). Whereas variations of concentrations by strains were much stronger with TNF-α than for MIP-2, there was a strong and significant correlation between MIP-2 dosages and mortality (r = 0.97; P < 0.005) and there was not any significant correlation between TNF-α dosage and mortality (r = 0.67).

**Conclusion:** Pertinent indicators of pathogenesis like the CS or MIP-2 concentrations could be useful in combination with mortality or in replacement of mortality to differentiate low and highly virulent strains in future studies. If these indicators had been used in replacement of mortality in our experimental design, half of mice (n = 100) would have not been used.

**P1012 Caenorhabditis elegans-based analysis for the host-pathogen interaction of Salmonella enterica subsp. enterica serovars isolated from indigenous vegetables and poultry meat in Malaysia**


*Caenorhabditis elegans* (C. elegans) have been widely used to study infections with promising results. Moreover, as its genome is surprisingly similar to that of humans (40% homologous), *C. elegans* becomes suitable as a simple host model.

**Objectives:** The increase of *Salmonella enterica* (S. enterica) occurrence in local indigenous vegetables and poultry meat can be potential health hazards. This study aimed to investigate the pathogenicity and persistent infection of various serovars *S. enterica* using *C. elegans* as a simple host model.

**Methods:** A total of *S. enterica* isolates (including 2 reference strains and 6 food sources) associated with 4 different serovars were tested. All *S. enterica* isolates were detected of virulence determinant by multiplex PCR. The virulence of *S. enterica* isolates in the *C. elegans* host model was evaluated by measuring the survival rate of worms fed on pure cultures of these isolates. Each assay was repeated 3 times for statistical analysis.

**Results:** Each *S. enterica* isolates under this study was found to possess up to 95% virulence genes. Result showed that different serovar have different mortality rate. The pathogenic *S. enterica* kills *C. elegans* faster than *E. coli* OPO50, which is the standard laboratory food strain. The time required for 50% *C. elegans* to die (TD50), which ranged from 3 to 4 days after ingesting various serovars of *S. enterica* compared to 17 days after ingesting the positive control strain *E. coli* OPO50. *S. enterica* shows similar persistency after 4 days of the infection which correlated to their TD50. Results from this study also revealed that the ability of *S. enterica* in killing of *C. elegans* correlates with its accumulation in intestine of the nematodes to achieve full pathogenicity.

**Conclusion:** The findings demonstrated that the virulence factors essential to mammalian pathogenesis also required for full pathogenicity in *C. elegans*.

**P1013 A gut colonisation model to study bacteriophages/bacteria relationships in vivo**


**Objective:** In order to study relationships between an established bacterial community and its specific bacteriophages, we developed an animal model using an enterogaegregative *Escherichia coli* strain known to persist in the mice gut for several weeks.

**Methods:** 7 week-old BALB/c mice were treated by streptomycin during 24 hours to eliminate most of intestinal facultative aerobic bacteria. The enterogaegregative *E. coli* pH5989 strain was isolated from stools of a patient who presented persistent diarrhoea was administered by oral gavage and the level of colonisation was followed by examination of the digestive tract over time. Bacteriophages were administered to the animals through drinking water during 24 hours.

**Results:** We first showed that gut colonisation with the strain 59989 was established within a few hours in the large intestine, and also interestingly in the small intestine which is quite rare for *E. coli* strains. New phages specific of the *E. coli* 59989 strain were isolated from Paris sewage water. A set of 3 phages were selected based on their host spectrum against a panel of 7 different *E. coli* strains. Interestingly each of the three phages belongs to a different family of the Caudovirales order.

A phage treatment (cocktail of the three phages) was given for 24 hours to 2 groups of animals: the first one was previously colonised by the *E. coli* strain and the second one was not. We first observed in mice faeces that phages remained for more than 18 days in the colonised group instead of 3 days in the control group suggesting that phages are able to multiply in vivo.

Then, we determined the bacterial content of the digestive tract at the end of the 24 h-phage treatment. We observed that the amount of bacteria remained stable in the large intestine but was significantly reduced in the small intestine.

**Conclusions:** This work allowed us to establish the conditions necessary to monitor phages/bacteria interactions in vivo when bacteria are part of a complex and stable community. These results suggest that the 3 phages are able to multiply in vivo and can clear partially the *E. coli* strain from the small intestine. These observations are of a particular interest in terms of phage therapy.

**P1014 Physical activity affects the risk of respiratory pneumococcal infection**

M. Pannacci*, V. Lucini, A. Caronno, F. Scaglione (Milan, IT)

**Objectives:** Many data shows that respiratory tract infection (RTI) represent one of the most common diseases spread among athletes,
whose frequency is about 7 times greater than a control subjects. A “J”-shaped model has been proposed to describe the relationship between physical activity and risk of RTI. Some observations in athletes lead to the hypothesis that moderate exercise will protect from infections while strenuous exercise will increase the susceptibility to RTI. However this hypothesis is not yet fully studied in validated experimental models. The purpose of this work was to evaluate the susceptibility to RTI in a model of experimental infection in mice subjected to different degrees of physical stress.

Methods: A validated experimental model of physical stress in that mice Balb/c were subjected to swimming for 1 h/day for 4 weeks, was used in this study. After the 1st (moderate) and 4th (chronic) week of exercise the animals were infected intranasally by inhalation with 1 × 10^8 CFU of *S. pneumoniae* ATCC49619 virulent for mice. The level of infection was evaluated 48 hours after infection; lungs were taken for histological analysis and bacterial counts.

Results: All controls and all chronic stressed mice resulted infected. However the pneumonia was markedly extended in the stressed group compared to controls. The bacterial size was significantly higher (p < 0.001) in the chronic stressed animals vs controls (4 × 10^6 ± 2.5 CFU/lung vs 3 × 10^5 ± 2 CFU/lung). In contrast, 30% of mice subjected to moderate exercise resulted infected only. The bacterial size was 2 × 10^6 ± 1.4 CFU/lung.

Conclusions: Data obtained experimentally confirmed that a prolonged strenuous exercise increases susceptibility as well as the severity of RTI; in contrast the moderate exercise results protective. The molecular basis of this phenomenon are still unknown. A key concept is that exercise has a direct effects on the immune system, leading to a change in innate response; recently it is becoming clear that moderate exercise may activate the innate immunological response while in intense and prolonged exercise the immunological response seems to be impaired to preserve immunity tolerance. Further research is warranted to address the molecular and cellular mechanisms of these observed effects.

**Objectives:** A recent clinical study demonstrated beneficial effects of adjuvant glycerol in children suffering from bacterial meningitis. The study demonstrates a significant reduction of severe neurological sequelae by glycerol. A collaborative study by the EMESG was initiated to investigate the mechanisms underlying the beneficial effect of glycerol in experimental pneumococcal meningitis.

**Methods:** Infant rats and adult mice were infected intracisternally with sterile saline containing *Streptococcus pneumoniae*. At 18 h and 24 h after infection, all animals received ceftriaxone (100 mg/kg). Furthermore, they were treated with glycerol or placebo (carboxymethylcellulose) starting from 18 h after infection. At 40 h after infection, animals were evaluated clinically. In infant rats, the brain was dissected to determine histomorphology and tissue density as an index of brain injury. Here, the effect of EPO on hearing loss and hippocampal neurogenesis were evaluated in an infant rat model of pneumococcal meningitis.

**Results:** In the infant rat model, glycerol reduced MPO-activity 24 h after infection and lowered the concentration of MPP-9 in the CSF at 40 h after infection. Hearing thresholds and histomorphology of the inner ear showed no significant differences between glycerol and placebo treated mice.

**Conclusions:** The reduced activity of MPO in the early phase indicates that less inflammatory cells (i.e. neutrophil) invaded the CSF. MMP-2 and particularly MPP-9 were shown to contribute to the development of brain injury in bacterial meningitis. Thus, the decrease of MPP-9 at 40 h after infection suggests a potential neuroprotective effect, possibly as a result of the reduction of pleocytosis. However, these effects of glycerol were not strong enough to translate into significant improvements in the infant rat and adult mouse model of pneumococcal meningitis.

**Objectives:** Garennoxacin (GRN), a des-fluoro (6)-quinolone, exhibits potent antibacterial activity against *S. aureus*, including MRSA, which causes severe skin infection in diabetic patients. We established a skin infection model with MRSA in diabetic mice and compared the efficacy of GRN to levofloxacin (LVX) and moxifloxacin (MXF).

**Methods:** Male C57BLKS/J lar--Leprdb/+Leprdb mice were used as type II diabetic mice. After shaving the hair on an area of the thigh under anesthesia, mice were infected by placing a 5 ul droplet containing ca. 10^6 CFU of MRSA F-3410 (MIC: GRN 0.013 mg/L, LVX 0.25 mg/L, MXF 0.0625 mg/L) on the thigh skin. Five mg/kg of each quinolone was administered orally once at 2 h after infection. Twenty-four h after infection, skin tissue was removed, and the viable cell count in skin was measured. At the same time, after fixation and staining with haematoxylin-eosin, histopathological observation was performed. The drug concentrations in thigh skin of mice infected with MRSA F-3410 were also measured by HPLC with a single oral dose of 20 mg/kg, and pharmacokinetic evaluations were performed by non-compartment analysis.

**Results:** The viable cell count in the skin (Log10 CFU/skin) of the GRN-treated group (3.41 ± 0.51) was significantly less than LVX- (6.18 ± 0.39*), MXF- (4.44 ± 0.31*) and non-treated (6.34 ± 0.40*) groups, (N = 10, Mean ± S.D., *: p < 0.001 vs. GRN). In the non-treated group, 24 h after infection, infiltration of inflammatory cells surrounding bacterial colonies reached the subcutaneous adipose tissue. After GRN treatment, although cell debris derived from inflammatory cells persisted on the skin surface, the inflammatory reaction was very slight. The AUCinf of GRN in the thigh skin at 20 mg/kg was 11.4 µg E/hg (N = 3, Mean) and the AUC/MIC ratio was 364, which was more than ten times greater than with LVX. The greater AUC/MIC ratio of GRN reflected its favourable therapeutic effect.

**Conclusions:** GRN is considered a valuable quinolone in the treatment of skin infections caused by *S. aureus*, including MRSA in diabetes.
and inner ear. BrdU cell density was determined in the dentate gyrus of the hippocampus.

**Results:** Hearing loss due to pneumococcal meningitis assessed 3 weeks after infection was significantly attenuated upon EPO treatment. While hearing threshold in infected, saline treated, animals was 81.1±17.1 decibels (dB, n=26), this threshold was reduced to 69.8±20.6 dB in infected, EPO-treated animals (p=0.04, t-test). In the hippocampus, EPO significantly increased neurogenesis. The rate of cell proliferation in the dentate gyrus 3 weeks after infection was 262±123 BrdU positive cells/mm² (n=5) in infected animals treated with EPO vs 148±21 cells/mm² (n=7) in infected animals treated with saline.

**Conclusion:** In experimental pneumococcal meningitis EPO attenuates hearing loss and increases cell proliferation in the neurogenic zone of the hippocampus. This pleiotropic effect may be due to a neuroprotective effect during the acute disease or increased cell regeneration in the inner ear and the hippocampus during the recovery phase.

**P1018 First experiences with N-chlorotaurine in a swine bronchopulmonary infection model**


**Objectives:** N-chlorotaurine (NCT), a new endogenous antiseptic, can be applied topically to different body regions. Recently we demonstrated the tolerability of inhalative NCT in the pig model.

In present studies NCT is investigated in a pig bronchopulmonary infection model.

**Methods:** In a pilot study anaesthetized pigs were infected by instillation of Streptococcus pyogenes through a catheter placed to the carina via the ventilation tube. An hour later, test solutions of 1% NCT (n=6) and 0.9% saline solution as a control (n=6), respectively, were inhaled. Applications were performed every hour within four hours, i.e. 4 inhalations in total, with 5 ml each. Lung function, blood oxygenation, and circulation were monitored. One hour after the last dosing the animals were euthanised, and bronchial alveolar lavage samples for bacterial cultures and lung samples for histology were removed.

**Results:** Tolerability of 1% NCT was very good, there were no hints for toxic side effects. Bronchial alveolar lavages revealed a higher bacterial count in the control (saline) pigs compared to NCT treated animals. Because of high standard deviations and the limited number of animals, this result was not yet significant. The same is true for oxygenation parameters where the values seemed to be better in the test group, too. Lung histology showed bacterial infection and inflammation as expected.

**Conclusion:** The good tolerability of inhalative NCT is confirmed also in the infection model. There are first hints for efficacy, which encourages further investigations.

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**P1019 Viral RNA replication is associated with changes in selenium in target organs of Coxsackie virus B3 infection**

Y. Molin*, P. Frisk, N.G. Ilbäck (Uppsala, SE)

**Objectives:** Selenium has been found to reduce replication of several viruses, both in vitro and in vivo, but it is unknown whether viral replication affects the host’s selenium balance in target organs of the infection. Further, studies of sequential changes in viral replication in target organs of infection are sparse.

**Methods:** In this study Balb/c mice were infected with Coxsackie virus B3 (CVB3) and sequential changes in viral replication (as determined by RT-PCR) were related to changes in selenium concentration (as determined by ICP-MS) on days 3, 5 and 7 of the infection in serum, heart, lung, liver, pancreas, kidney, spleen, intestine and brain.

**Results:** After an initial viral peak on day 3, viral load drastically decreased in all organs, i.e. by >99% (serum), 97% (lung), 98% (liver), 60% (pancreas), 95% (kidney) and 93% (spleen), except in the intestine, intestine and brain, where viral load in fact tended to increase after day 3. Selenium decreased in all organs except the heart. Moreover, selenium was negatively correlated to viral load in serum, liver, pancreas and intestine.

**Conclusion:** These findings give evidence that selenium is directly involved in the replication of CVB3.

**P1020 Intravital profiling of pramiconazole against a panel of moulds and yeasts and in vivo activity against Microsporum canis and Candida albicans**

K. de Wit*, A. Matheussen, E. Béniré, G. Van Minnebruggen, P Cos, L. Maes (Antwerp, Geel, BE)

**Objectives:** In vitro and in vivo evaluation of a novel triazole antifungal, pramiconazole, (Stiefel Laboratories Inc., formerly Barrier Therapeutics Inc.) in comparison to two leading dermatomycological reference compounds israconazole and terbinafine.

**Methods:** The in vitro susceptibility (IC50-values) of several moulds and yeasts was evaluated in a microplate assay within the dose range 64–0.03125 micromolar, adopting two-fold dilution series. In each plate, positive, negative and reference controls were included. The dye resazurin was used as viability indicator, allowing spectrophotometric reading. Next, the clinical efficacy of pramiconazole was evaluated after topical and oral administration in the guinea pig model with Microsporum canis and the rat vulvovaginal model with Candida albicans. To our knowledge, pramiconazole had never been tested in the latter model. In both models, drug formulations were PEG400 for oral dosing and a mixture of PEG400 + PEG1500 for topical application. Skin lesions were scored using a semi-quantitative scoring system based on severity and lesion size. Intravaginal infection burdens were determined by plate-counting vaginal swabs taken at different days post-infection.

**Results:** Pramiconazole exhibited good in vitro activity against the dermatophytes M. canis (IC50 0.17±0.02 micromolar), Trichophyton rubrum (IC50 0.25±0.18 micromolar) and T. mentagrophytes (IC50 0.08±0.10 micromolar) and against the yeasts C. albicans (IC50 0.03±0.01 micromolar) and C. parapsilosis (IC50 0.85±0.78 micromolar). In addition, high potency was present against Cryptococcus neoformans (IC50 0.20±0.33 micromolar). In contrast to itraconazole and terbinafine, no activity was observed against Sporothrix schenckii and Aspergillus fumigatus (IC50 >64 micromolar). In both animal models, pramiconazole was comparable or slightly better than itraconazole and clearly superior to terbinafine. In the vulvovaginal C. albicans model, superior activity of pramiconazole to fluconazole was also found.

**Conclusion:** Pramiconazole is a promising new triazole antifungal showing comparable in vitro potency to terbinafine against dermatophytes. Pramiconazole was associated with faster healing of skin lesions and faster clearance of C. albicans vaginal infection than comparator drugs, suggesting a more advantageous pharmacokinetic profile.

**P1021 Reduction in serum lipid parameters by incorporation of Lactobacillus plantarum A7, native strain in mice diet**

H. Fazeli*, J. Moshaghiian, M. Mirlohi, M. Shirzadi (Isfahan, IR)

**Objectives:** Yearly many deaths occurred because of coronary heart disease and there is relationship between hyperlipidaemia and its incidence. Drugs used for reduction of cholesterol and other serum lipids have very adverse effects and imposed very expenses to patients. Therefore new ways presented for reduction of serum lipids such as lactic acid bacteria. Researches shows a variable range of cholesterol lowering effects for specific strains of lactic acid bacteria and routine use of them still is not complete and need more research to elucidating the geographical events on usefulness of them. So the aim of this study was to assess the effects of native L. plantarum strain on mice blood lipid profile, when it introduced in their daily diets and compromised it with other probiotics.

**Methods:** Lactobacillus spp isolated from faecal samples of 11 infants 3–21 month. More identification performed by PCR and probiotic aspects defined. Lactobacillus plantarum A7 due to its superior bile
resistance among several Lactobacillus spp was selected for evaluation of its effect on cholesterol reduction. 16 Male Balb/c mice weighing 25 to 30g were fed with a high cholesterol regimen diet. After 14 days, serum of all samples analyzed for lipid parameters. In the next fourteen days mice randomly divided to two groups, one received 10 8 CFU/ml of lactobacillus plantarum by gavage and second don’t received any bacteria. Then again serum lipid parameters measured.

Results: In treated group pre intervention means of Cholesterol, triglyceride, HDL and LDL was 101.3, 105.1, 40.9 and 35.6 respectively. According to statistical analysis no significance differences obtained between Treated and Control groups (P= 0.87). After intervention in treated group mean of Cholesterol, triglyceride, HDL and LDL was 92, 97.7, 46 and 30.4 respectively. In control group mean of Cholesterol, triglyceride, HDL and LDL was 106.3, 146.6, 39.1 and 42.4 respectively. Comparison of lipid profile in treated and control group showed cholesterol, triglyceride and LDL had reduced in treated group significantly (<0.05, p=0.001, p=0.015 respectively) but for HDL this difference not significant (p=0.19).

Conclusion: Our results showed oral administration of L. plantarum A7 lowered harmful serum lipids without any pathogenic side effects. The results of this study and comparison of it with others showed in use of probiotics and its biological products geographical aspects must be considered.

P1022 24-nor-ursodeoxycholic acid as novel therapeutic strategy for inflammation-induced liver fibrosis in a murine model of Schistosoma mansoni infection


Objectives: Vigorous granulomatous and fibrotic reactions to parasite eggs specify the pathological picture of Schistosoma mansoni infection as one the leading causes of liver fibrosis and portal hypertension. Current antihelminthic therapy is ineffective once hepatic fibrosis is established. The aim of our study was to test 24-nor-ursodeoxycholic acid (norUDCA) as a novel therapeutic strategy for liver fibrosis since it is known to have great anti-inflammatory and/or anti-fibrotic effects in a mouse model of biliary fibrosis.

Methods: Adult NMRImice were infected with 50 S. mansoni cercariae. Hepatic fibrosis was studied at two time points (8, 24 weeks post infection, pi). To test effects of bile acids, mice received a 0.5% bile acid diet (norUDCA and UDCA) for 4 weeks. Effects on liver histopathology (sirus red, a-smooth muscle actin, a-SMA; keratin 19, K19), serum liver biochemistry and inflammation (CD4, CD11b) as well as expression of fibrosis-related genes (TNF-α, IFN-γ, iNOS, TGF-β, α-SMA) were compared to untreated controls.

Results: S. mansoni infected mice showed a time-dependent increase in total hepatic hydroxyproline content (7-fold, 24 weeks after infection, pi, <0.05). No changes in serum liver parameters were detectable. Expression levels of fibrosis-related genes (col1a2, TGF-β1, iNOS) were elevated during infection, peaking around 8 weeks. Dysbalance of mRNA expression of matrixmetalloproteinase-2 (MMP-2) and tissue inhibitor of matrixmetalloproteinase-1 (TIMP-1) was established after 8 weeks (12-fold and 38-fold increase, respectively, p<0.05). Bile-duct proliferation became prominent after 24 weeks. NorUDCA but not UDCA reduced hydroxyproline content and a-SMA expression (1.5-fold and 2-fold, respectively, p<0.05). Liver histopathology revealed a reduction in hepatic fibrosis and granuloma size (33% compared to untreated controls, p<0.05). IHCs showed a decrease in inflammatory cell count. In contrast, conventional UDCA did not improve liver histology.

Conclusions: This study demonstrates (i) a time-dependent development of non-cholestatic liver fibrosis in S. mansoni infected mice, (ii) anti-inflammatory properties of norUDCA and (iii) beneficial effects of norUDCA on granuloma size and hepatic fibrosis, therefore qualifying norUDCA as a promising drug for the treatment of non-biliary liver fibrosis.

P1023 Vancomycin versus linezolid in the therapy of experimental methicillin-resistant Staphylococcus aureus meningitis

S. Calik, T. Turhan, T. Yurtsever, O. Sipahi*, C. Buke (Izmir, TR)

Objectives: The aim of this study was to compare the antibacterial efficacy of vancomycin and linezolid in a rabbit model of methicillin-resistant Staphylococcus aureus (MRSA) meningitis.

Methods: New Zealand white rabbits weighing 2–2.5 kg were anaesthetized by ketamine (55 mg/kg) and xylazine (5 mg/kg) before each intraventricular intervention including induction of meningitis and CSF sampling. Meningitis was induced by intracisternal inoculation of MRSA (ATCC 43300) strain. After 16th incubation time and development of meningitis, rabbits were separated into three groups. Group 1 was given vancomycin 20 mg/kg every 12h, Group 2 was given linezolid 20 mg kg every 12h, and Group 3 was control. Cerebrospinal fluid bacterial counts were measured at 16 and 40h. Bacterial concentrations in CSF were measured at 16th, 40th h. of the study by plating undiluted and serial 10-fold and 100-fold dilutions of CSF (10 μL) on 5% sheep blood agar and incubated at 37°C for 24h. Bacterial response was evaluated in three categories; full response, sterilisation of CSF, partial response, any decrease in bacterial count; and bacteriological failure, a stable or increased bacterial count. Data were evaluated by SPSS 11.0 package program using Mann-Whitney U test, Kruskal Wallis test and Fisher’s χ² test. A p-value less than 0.05 was considered significant. The study was approved by the local ethical committee on animal studies.

Results: At the beginning of the study, 45 rabbits were inoculated with bacteria, of which 33 were alive at the end of 16h. Bacterial counts were similar in all groups at 16h. At 40h bacterial counts were similar in vancomycin and linezolid groups (p<0.05), and both counts were lower than control group (see table, p<0.05). During the study, mortality was similar among three groups (2/11 in gr. 1, 6/11 in gr. 2 and 5/11 in gr. 3, p=0.192). At the end of the study, rates of full (2/11 in gr. 1, 2/11 in gr. 2), partial (5/11 in gr. 1, 1/11 in gr. 2) and full or partial bacteriological response (7/11 in gr. 1, 3/11 in gr. 2) were similar in two treatment groups (p>0.05). The decrease in bacterial counts in vancomycin and linezolid groups at 40h was also similar (−2.860 ± 4.495 versus −0.724±4.360 log10 CFU/mL, P>0.05).

Conclusions: These results suggest that linezolid is not inferior to vancomycin in the treatment of MRSA meningitis in experimental rabbit model. Additional data should confirm in advance of clinical trials to assess efficacy in humans.
guinea pig infection model with subcutaneously implanted Teflon cages was used. DAL concentrations were determined in sterile cages after a single dose of 10, 20 and 40 mg/kg i.p. Cage-associated infection was established by percutaneous injection of MRSA (5 × 10^6 CFU/cage). 3 days after infection, i.p. treatment was started (3 animals/treatment regimen): DAL 40 mg/kg single dose, RIF 12.5 mg/kg/12 × 4 days and their combination. Bacterial titer in cage fluid was determined 5 and 9 days after treatment start. Then were animals sacrificed and cages cultured in TSB for 48 h. Cure rate (%) is the proportion of cage cultures without MRSA. Positive cage cultures were tested for development of RIF resistance.

**Results:** DAL MIC was 0.06 µg/mL and MBC was >20 µg/mL. Time-kill curve studies showed a reduction of bacterial load >99.9% after 48 h with DAL concentrations >5 µg/mL in logarithmic and ≥0.157 µg/mL in stationary growth. After single dose of 10, 20 and 40 mg/kg DAL, in cage fluid Cmax was 4.6, 18.2 and 34.9 µg/mL at 10–12 h; AUC was 150, 926, 3018 µg h/mL respectively. Bacterial counts in cage fluid at start of treatment ranged from 7.0–7.3 log cfu/mL. Addition of RIF significantly improved the efficacy of DAL on planktonic bacteria in cage fluid during (p = 0.06) and after treatment (p < 0.001) (Figure). The cure rate with DAL alone was 8%, which was improved in combination with RIF to 33% (p < 0.05), which did not differ from the RIF monotherapy, but reduced the frequency of RIF resistant cage cultures from 38% to 25%.

**Conclusions:** DAL alone reduced 0.5–1.1 log cfu/mL MRSA in cage fluid and had a cure rate of 8%; DAL combined with RIF reduced >5 log cfu/mL MRSA in cage fluid and achieved a 33% cure rate. The combination DAL plus RIF deserves further studies to determine the optimal dosing for implant-associated MRSA infections are needed.

**Objective:** Previous studies have shown the suboptimal activity of vancomycin (VAN) in the treatment of severe infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) strains with MIC > 1 mg/L. Also, the efficacy of linezolid (LZD) in clinical MRSA pneumonia appears to be higher than that obtained with VAN. Moreover, there are controversial results about the efficacy of cotrimoxazole (COT) in the treatment of severe infections caused by MRSA. The aim of the study is to compare the efficacy of VAN, LZD, and COT, in a murine pneumonia model, caused by MRSA.

**Methods:** MIC of VAN, LZD, and COT were determined for 2 clinical MRSA strains (MRSA30 and MRSA33). The bactericidal activity (time–kill curves; 1, 2 and 4 × MIC) were studied for both strains. In vivo: pneumonia model in neutropenic C57BL/6 mice (cyclophosphamide 150 mg/kg) was used, with an intratracheal inoculum of 8–9 Log10 cfu/mL. Pharmacokinetic/pharmacodynamic (PK/PD) parameters (Cmax [mg/L]; AUC [mg h/L]; t1/2 [h]; t½-MIC [h]; AUC/MIC) were determined in serum after a single dose of 30 mg/kg and 110 mg/kg of VAN, 30 mg/kg of LZD, and 10.50 mg/Kg of COT on untreated mice. For the experimental pneumonia, mice were randomly grouped in untreated (CON), VAN (30 mg/kg) and VAN (110 mg/kg) every 6 hours, LZD (30 mg/kg) every 6 hours, and COT (10/50 mg/Kg) every 4 hours. Analyzed variables (after 72 h treatment): bacterial lung concentration (Log10 cfu/g), negative blood cultures (%), survival (%). Statistical analysis: ANOVA, post hoc test, and Chi-square tests.

**Results:** MIC (mg/L) for MRSA30 and MRSA33 were: VAN = 1, LZD = 4, and COT = 0.13. Bactericidal activity (3 × Log10 in bacterial reduction): MRSA30, VAN (2 × MIC, 4 × MIC); MRSA33, COT (4 × MIC), VAN (4 × MIC). PK (Cmax, AUC; t1/2): VAN (30 mg/kg) 35.54, 35.08, 0.42; VAN (110 mg/kg) 97.79, 111.36, 0.57; LZD (30 mg/kg) 25.31, 36.22, 0.93; COT (10/50 mg/kg) 1.89, 1.87, 1.69. PD (t½-MIC; AUC/MIC): VAN (30 mg/kg) 1.85, 35.08; VAN (110 mg/kg) 3.9, 111.36; LZD (30 mg/kg) 2.72, 9.06; COT (10/50 mg/kg) 1.45, 14.38.

**Conclusions:** Overall, linezolid was the most efficacious antimicrobial treatment in the experimental pneumonia caused by MRSA. In order to reach an optimal activity, vancomycin needs a high dose to obtain an AUC/MIC > 24 h ratio above 400, when its MIC is 1 mg/L. The results showed that cotrimoxazole is as efficacious as vancomycin at high dose, which suggests that it could be used as therapeutic alternative in the pneumonia caused by MRSA.

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**P1025** Antibacterial activity of NXL103 (linopristine-flopristin) against intracellular *Staphylococcus aureus* and efficacy following per oral administration in murine models of systemic infection

**J. Paez**, P. Lecasson, A.M. Giraud, L. Lacaillede (Romainville, FR)

**Objectives:** NXL103 (linopristine-flopristin) is an oral streptogramin which was recently evaluated in a Phase II clinical trial. Activity has been observed against a wide variety of Gram-positive pathogens as well as *Haemophilus influenzae* and *Moraxella catarrhalis*. We here report in vitro antibacterial activity against intracellular *Staphylococcus aureus* (SA) and in vivo efficacy in murine infection models.

**Methods:** SA strains were opsonised in medium 199 containing 10% guinea-pig serum and incubated with 2774 macrophages followed by inactivation of extracellular bacteria, and incubation with antibiotic (2–8 × MIC, 1–4 h). Surviving intracellular bacteria were released by sonication and enumerated by plate counting. Septicaemia was established in ICO:OF1 mice by intraperitoneal injection of approximately 7 × 10^9 CFU in 7.5% mucin suspension following growth in BHI broth. Efficacy in the murine thigh model was evaluated following injection of the right thigh muscle with 3 × 10^5 CFU of bacteria. Antibiotics were administered either orally (p.o.) or subcutaneously (s.c.) at 1 and 6 h post-infection, and the 50% effective dose (ED50) was determined.

**Results:** NXL103 reduced susceptible SA counts from 1.25 to 1.96 log10 within 4 h at concentrations from 1 × to 8 × MIC. Counts of two intracellular MLSB-constitutitively resistant SA strains were reduced by 1.41 to 1.58 log10 when infected macrophages were treated with 8 × MIC. This antibacterial effect of NXL103 was superior to that observed with clarithromycin or vancomycin. In the murine models of systemic bacterial infection, the NXL103 ED50 (p.o.) ranged from 20–60 mg/kg for the methicillin-susceptible and methicillin-resistant MLSB strains tested. Oxacillin and clarithromycin activities (p.o.) were similar or inferior to NXL103 depending on the strain susceptibility, and the vancomycin ED50 (s.c.) was 2.2–2.4 mg/kg for treatment of septicaemia, and 17–70 mg/kg in the thigh model.

**Conclusion:** NXL103 kills intracellular bacteria in infected macrophages in vitro and is efficacious against *S. aureus*, including drug-resistant strains, following oral administration to infected mice.
Objectives: Bacteremia and systemic complications contribute to the development of brain damage and may be directly responsible for up to half of all fatal cases of pneumococcal meningitis.

Methods: Using an adult rat pneumococcal meningitis model, the impact of accompanying bacteremia on the development of hippocampal injury was studied in the following groups: Meningitis controls (n = 11), meningitis with early onset bacteremia from concomitant iv infusion of pneumococci (n = 9), meningitis with attenuated bacteremia resulting from iv injection of serotype-specific pneumococcal antibodies (n = 10), and uninfected controls (n = 6).

Results: Degree of hippocampal neural apoptosis is shown in the Figure. Pneumococcal meningitis resulted in significantly higher degree of neural apoptosis 0.22 (0.18–0.35) as compared to uninfected controls (0.02 (0.00–0.02), P = 0.0003). Among meningitis groups, attenuation of bacteremia by antibody treatment resulted in significantly reduced apoptosis (0.08 (0.02–0.20), P = 0.01).

Conclusion: Our results suggest that accompanying bacteremia has a significant role in development of hippocampal injury during pneumococcal meningitis.

Efficacy of oritavancin in the rat haematogenous pneumonia model


Objectives: Oritavancin (ORI) is a semi-synthetic lipoglycopeptide with bactericidal activity against Gram-positive cocci including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin (VAN)-resistant *S. aureus*. ORI was highly active in the mouse pneumonia model of *Streptococcus pneumoniae* infection. Here, we have simulated blood-borne staphylococcal pneumonia and have investigated the efficacy of ORI in the rat haematogenous (HP) model of infection caused by MRSA.

Methods: Infection was established in CD rats (n = 5/group) by injecting 1.2×10⁷ colony-forming units (CFU)/mL of MRSA strain NRS123 (USA 400) (ORI MIC=0.06 mg/L) emmeshed in 0.08% agar beads (as described by Sawai et al., 1997; Infect. Immun 65:466). The rat HP model was established by injecting 0.5 mL of agar beads bacteria suspension intravenously (i.v.). To assess the efficacy of ORI at 50 mg/kg i.v., the rats were treated from Day 1 to Day 6 PI once daily. The ORI treatment was compared to VAN (100 mg/kg, subcutaneous (s.c.), daptomycin (DAP) (50 mg/kg, s.c.) and nafcillin (NAF) (150 mg/kg, s.c., twice daily). The lungs and spleen of the infected rats were harvested on day 1 and day 6 or 7 post-infection (PI) and homogenised to determine the bacterial burden. The bacterial densities were expressed in mean Log CFU/organ ± standard deviation and the statistical calculations were performed according to the Kruskal-Wallis and Mann-Whitney U tests by using StatsDirect software. P-values below 0.05 were considered significant.

Results: The rat HP infection was successfully established and stable over 7 days. On day 6 PI, 5.41±0.91 Log CFU were recovered in lungs, while 1.94±0.25 Log CFU/spleen were recovered showing that ORI was efficacious in the rat HP model. All treatments (except NAF) decreased significantly the CFU counts in lungs compared to untreated. MRSA densities on day 7 PI were 7.68±0.42, 4.10 ± 0.59, 5.26±0.65, 6.39±0.43 and 7.32±0.47 Log CFU/lung for untreated, ORI, DAP, VAN and NAF, respectively; p = 0.008, except NAF. The ORI regimen also significantly decreased bacterial densities compared to other comparators (p< 0.036 for ORI vs DAP, VAN and NAF).

Conclusion: ORI was efficacious in the rat HP model. Moreover, ORI had greater efficacy than all the comparators as tested. These results suggest that ORI might be useful for the treatment of human blood-borne pneumonia infections.

In vitro antibacterial susceptibility of Gram-negatives

Investigation of carbapenem heteroresistance among susceptible *Acinetobacterbaumanni*

E. Neou*, A. Ikonomidis, A. Katsiaflaka, A. Vasdeki, A. Maniatis, A. Tsakris, S. Pourounas (Larissa, Athens, GR)

Objectives: Carbapenem heteroresistance in *Acinetobacter baumannii* has been described previously and might threaten carbapenem activity. The present study aimed to investigate the heteroresistant phenotype among apparently susceptible *A. baumannii*, against which carbapenems may be used and heterogeneity might affect treatment outcome.

Methods: Fourteen carbapenem-susceptible *A. baumannii* clinical isolates were recovered during a six-month period. Carbapenem MICs were tested by agar dilution. Pulsed-field gel electrophoresis for clonality and PCR for known carbapenemase-genes were performed. Heteroresistant subpopulations were revealed by population analyses and carbapenem MICs of the subpopulations were estimated after subcultures in drug-free medium, to investigate the heteroresistance stability. Time- killing studies utilising meropenem at 4 mg/L were also performed.

Results: Imipenem and meropenem MICs of the native isolates ranged from <0.25 to 6 mg/L and <0.25 to 4 mg/L, respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses (mg/kg)</th>
<th>No.</th>
<th>Log cfu/g lung (mean±SD)</th>
<th>Negative blood culture (%)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MRSAA30</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>CON</td>
<td>14</td>
<td>8.93±0.78</td>
<td>3 (21.4)</td>
<td>12 (85.7)</td>
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</tr>
<tr>
<td>VAN</td>
<td>14</td>
<td>6.67±3.01</td>
<td>9 (64.3)</td>
<td>13 (92.9)</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>13</td>
<td>3.25±1.59</td>
<td>13 (100)</td>
<td>10 (76.9)</td>
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</tr>
<tr>
<td>LZD</td>
<td>16</td>
<td>2.87±1.86</td>
<td>15 (93.8)</td>
<td>16 (100)</td>
<td></td>
</tr>
<tr>
<td>COT</td>
<td>15/50</td>
<td>3.18±2.04</td>
<td>15 (100)</td>
<td>15 (100)</td>
<td></td>
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<tr>
<td><strong>MRSAA33</strong></td>
<td></td>
<td></td>
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<tr>
<td>CON</td>
<td>15</td>
<td>8.62±0.72</td>
<td>6 (40)</td>
<td>10 (66.7)</td>
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<tr>
<td>VAN</td>
<td>15</td>
<td>5.76±2.43</td>
<td>10 (66.7)</td>
<td>15 (100)</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>16</td>
<td>3.97±1.52</td>
<td>16 (100)</td>
<td>12 (75)</td>
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</tr>
<tr>
<td>LZD</td>
<td>15</td>
<td>1.59±1.40</td>
<td>14 (93.3)</td>
<td>15 (100)</td>
<td></td>
</tr>
<tr>
<td>COT</td>
<td>15/50</td>
<td>3.08±2.49</td>
<td>13 (86.7)</td>
<td>15 (100)</td>
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</tr>
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</table>

*p < 0.05 respect CON; b p < 0.05 respect VAN (30 mg/kg); c p < 0.05 respect VAN (110 mg/kg); d p < 0.05 respect COT.*
The isolates belonged to nine genotypes carrying solely the intrinsic blaOXA-51 gene. Population analysis assays for imipenem had colonies grown in concentrations from 8 to 24 mg/L for half of the isolates, whereas the rest remained within the susceptible range. Impenem-heteroresistant colonies returned to carbapenem susceptibility after subcultures in drug-free medium. Population analyses for meropenem revealed colonies grown from 8 to 32 mg/L for all isolates except one (AB13). These colonies retained elevated meropenem but not imipenem MICs after subcultures in drug-free medium. In time-killing assays using meropenem, four isolates (incl. AB13) were killed in a time-dependent manner, while in ten isolates the initial bactericidal effect was followed by substantial re-growth after 9 to 12 hours (three isolates) or 24 h (seven isolates) of incubation. Six of the fourteen patients were treated with meropenem and in five patients (three of whom died due to A. baumannii bacteraemia), whose isolates had significant re-growth in time-killing, meropenem treatment was not efficient. Notably, the patient successfully treated by meropenem was infected from an isolate sufficiently killed in vitro.

**Conclusion:** Our findings indicate that meropenem susceptible *A. baumannii* have the potential to produce heteroresistant subpopulations under meropenem pressure. Should these subpopulations be selected under inappropriate meropenem treatment they may subsequently affect patient outcome.

**References:**

P1030 In vitro activities of various antimicrobials alone and in combination with imipenem against carbapenem-resistant *Acinetobacter baumannii* blood isolates

**WH. Sheng* (Taipei, TW)**

The prevalence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) related infections continue to increase, however, therapeutic options for treatment of infections with CRAB have limited. In this study, in vitro synergism using checkerboard titration and time-kill method among amikacin, ciprofloxacin, colistin (polymyxin E), sulbactam and tigecycline alone and in combination with imipenem were investigated among 15 epidemiologically different CRAB blood isolates. THP-1 cells were added as antimicrobial-phagocyte synergistic model. Colistin (MIC90: 1 μg/mL, susceptible 95.8%) and tigecycline (MIC90: 2 μg/mL, susceptible 91.7%) were the most the two active agents against CRAB, followed by minocycline (susceptible 87.5%), amikacin (58.3) and sulbactam (susceptible 47.7%). In checkerboard titration, synergism existed with imipenem while combined with all test agents, including colistin (33.3%), tigecycline (33.3%), amikacin (26.7%), sulbactam (26.7%) and ciprofloxacin (13.3%). In time kill method, antimicrobial synergy and bactericidal effects were found in amikacin (66.7% and 53.3%), ciprofloxacin (33.3% and 40%), colistin (80% and 73.3%), tigecycline (66.7% and 40%) and sulbactam (53.3% and 46.7%) while combined with imipenem, respectively. The results of antimicrobial synergistic effects were not affected by existing of phagocytes (THP-1 cells). Of six CRAB isolates with high minimal inhibitory concentration (MIC) of imipenem (>32 μg/mL), none had antimicrobial synergy by checkerboard titration. In summary, our results demonstrate synergism between imipenem in combination with various antimicrobial agents, such as colistin, tigecycline, amikacin, sulbactam and ciprofloxacin, against *A. baumannii* isolates with low or intermediate resistance to carbapenem. For those serious infections caused by highly imipenem-resistant *A. baumannii* (MIC > 32 μg/mL), newer antimicrobial combination, such as amikacin, colistin and tigecycline should be considered. Surveillance of carbapenem resistance and known the susceptibilities of prevalent *A. baumannii* strains, might provide the information to develop an appropriate empirical therapy for CRAB infections.

**References:**

P1031 Multidrug-resistant *Acinetobacter: is colistin still effective?**

O. Koseoglu Eser, A. Ergin* (Ankara, TR)

**Objectives:** *Acinetobacter* is a primary nosocomial pathogen causing ventilator-associated pneumonia, meningitis, endocarditis and sepsis, especially in intensive care units (ICUs). The study was performed to investigate the antibiotic susceptibility and metallo-β-lactamase production of *Acinetobacter* isolates in patients hospitalised in Hacettepe University Train Hospital, Ankara, Turkey in a one year period.

**Methods:** The microorganisms have been isolated from clinical specimens of patients with respiratory and bloodstream infections. In vitro activity of imipenem (IM), meropenem (MER), ceftazidime (CAZ), ciprofloxacin (CIP) and aztreonam (AZT) in clinical *Acinetobacter* species isolated was evaluated by microdilution test. Each isolate was also tested for metallo-β-lactamase (MBL) production by using IMP and EDTA combined disk diffusion test. Antimicrobial susceptibility testing was performed in MDR isolates for colistin by microdilution, and for amikacin (AN), piperacillin-tazobactam (PIP-TAZ), cefepime (FEP), ceftriaxone (CRO), tetracycline (TET), trimetoprim-sulfamethoxazole (SXT), mezlocillin (MEZ) by disk diffusion method. All antimicrobial susceptibility tests were done according to CLSI guidelines.

**Results:** Among nonduplicate 124 isolates, 72 were *Acinetobacter baumannii*, 52 were *A.iovelli*. Forty five (36.3%) of isolates were from patients in ICUs. MIC50 and MIC90 (μg/mL) values of isolates were found 32 and 128 (IM), 16 and 32 (MER), 128 and 256 (CIP), 64 and 256 (CAZ), 128 and 256 (AZT), respectively. Only forty three (34.7%) isolates were susceptible to IMP. Forty six (51.6%) were positive for IMP-EDTA combined disk test. Overall, 51 (64.1%) *Acinetobacter* spp. were found to be resistant to ≥3 antibiotics belonging to different antimicrobial classes and defined as MDR. Colistin MIC50 and MIC90 values were 2 and 8 and resistance was found 27.5% in MDR isolates. The resistance rates for AN, PIP-TAZ, FEP, CRO, TET, SXT and MEZ were 80.4, 98.0, 92.2, 100.0, 100.0, 86.3, 86.3% respectively.

**Conclusion:** The rate of resistance against antimicrobial agents tested was high in our isolates. Colistin was the only active drug in high resistant isolates. Although there is a need for new drug development against multidrug resistant *Acinetobacter baumannii* isolates, the study suggests that colistin may still be an alternative therapeutic option in MDR *Acinetobacter baumannii* isolates.
of Osalen® was noted. It appeared that Phe-Arg-b-naphthylamide affected the susceptibility of majority of tested strains to nalidixic acid and – non-antibiotics drugs.

**Conclusions:** Some drugs containing as an active substance: alendronate, ticlopidine, amitrypilnine and ribavirin substance showed a direct antimicrobial activity. Moreover, these drugs are also substrates for the efflux pumps in some Gram-negative rods. Unlike salicylate, these drugs do not induce efflux-mediated resistance to nalidixic acid.

### Comparative susceptibility of European Gram-negative pathogens to cephotibrole, cefazidime and cefepime

**H. Seifert, M. Dyrenen, A. Quintana, J. Launfer, P. Okolo, I. Morrissey**

(Cologne, DE; Winchester, UK; Raritan, US; Baar, CH; Fordham, UK)

**Objectives:** Cephotibrole (BPR) is a novel cephalosporin with bactericidal activity against both Gram-positive and Gram-negative bacteria, including activity against methicillin resistant *S. aureus* (MRSA). The Cephotibrole Local Antibiotic Susceptibility Surveillance Study (CLASS) compared the susceptibility of common Gram-negative pathogens causing serious infections in hospitalised patients. Here we report the comparative activity of BPR, cefazidime (CAZ) and cefepime (CFP) against Gram-negative pathogens (GNP).

**Methods:** 1,271 GNPs (including 366 *E. coli*, 299 *P. aeruginosa*, 237 *Klebsiella* spp., 223 *Enterobacter* spp. and 140 *H. influenzae* were collected from 32 centres in Austria, Germany, Ireland, Poland, Switzerland and UK during 2008 from hospitalised patients with CSSTI, bloodstream infections or nosocomial pneumonia including VAP. MICs were determined at each centre using Etest methodology.

**Results:** Against all GNPs combined, all three agents had an MIC90 of 4mg/L but BPR had the lowest MIC50 of 0.06mg/L compared with 0.125mg/L for CFP and 0.25mg/L for CAZ. Against *E. coli*, *H. influenzae* and *Klebsiella* spp., BPR had a lower MIC90 than CAZ and CFP (0.125, 0.25 and 0.5mg/L vs. *E. coli*: 0.125, 0.25 and 0.25mg/L vs. *H. influenzae*: 0.125, 1 and 0.25mg/L vs. *Klebsiella* spp. respectively). Against *Enterobacter* spp., BPR had a lower MIC90 (4mg/L) than CAZ (128mg/L) but two-fold higher than CFP (2mg/L). Against *P. aeruginosa*, BPR, CAZ and CFP MIC90 were 16mg/L, 8mg/L and 8mg/L respectively.

**Conclusion:** BPR showed activity comparable to CFP but generally more active than CAZ against a range of GNPs.

### Comparative susceptibility of European Gram-negative rods to doripenem, imipenem and meropenem

**R. Matters, M. Morgan, P. Nordmann, A. Quintana, J.M. Lasureffer, D. Cooper, I. Morrissey**

(Marburg, DE; Exeter, UK; Le Kremlin-Bicêtre, FR; Raritan, US; Baar, CH; Fordham, UK)

**Objective:** Doripenem (DPM) is a new carbapenem recently introduced into Europe. The Comparative Activity of Carbapenem Testing Study (COMPACT) compares the susceptibility of common Gram-negative rods (GNR) causing serious infections in hospitalised patients to DPM, imipenem (IMP) and meropenem (MPM).

**Methods:** 1,162 GNPs (542 *Pseudomonas* spp, 504 *Enterobacteriaceae* and 111 other GNRS were collected from 21 centres in France, Germany, Ireland and UK during 2008. DPM, IMP and MPM MICs were determined using Etest methodology and susceptibility interpreted according to CLSI break points for IMP and MPM, and FDA breakpoints for DPM.

**Results:** The MIC90 against all isolates was 2, 4 and 32mg/L for DPM, MPM and IMP. *Enterobacteriaceae* (43% *E. coli*, 22% *Klebsiella* spp., 13% *E. cloacae*) were highly susceptible to all 3 carbapenems with MIC90 of 0.125mg/L for DPM & MPM but 0.5mg/L for IMP. DPM had the lowest MIC90 against *Pseudomonas* spp. at 8mg/L with MPM at 16mg/L and IMP at ≥ 64mg/L. Resistance to DPM, IMP and MPM was seen in 7.3%, 11.8% and 6.8% of all isolates respectively and resistance to DPM (10.9%) and MPM (9.8%) in *Pseudomonas* spp. was similar and approximately half that for IMP (19.2%).

**Conclusion:** DPM showed excellent activity against GNRs being generally more active than IMP and at least as good as MPM. Against *Pseudomonas* spp., DPM was more active than both IMP and MPM, and DPM/MPM resistance in *Pseudomonas* spp. was similar and lower than that for IMP.

### Temocillin in vitro activity against ESBL- and AmpC-producing Gram-negative bacteria isolated in tertiary-care hospital

**L. Naumisak*, A. Samet**

(Gilanak, PL)

**Objectives:** The aim of the study was to determine temocillin in vitro activity against ESBL- and derepressed AmpC-producing Enterobacteriaceae isolated in tertiary care hospital in Poland

**Methods:** Fifty Enterobacteriaceae isolates from 2008 from which 31 produced ESBL and 19 produced derepressed AmpC enzyme were selected for temocillin activity determination. Modified double disk test (DDT) was done with cepheixime, cefotaxime and amoxicillin-clavulanic acid disks for ESBL detection. Derepressed AmpC isolates were selected based on negative result in DDT, resistance to cefotaxime and ceftazidime and susceptibility to cefepime. Disk diffusion method with 30ug temocillin (Neosensitabs, Rosco) was used and inhibition zones were recorded.

**Results:** There were 22 *Enterobacter cloacae* (16 AmpC), 12 *E. coli*, 7 *ESBL Klebsiella* spp., 4 Proteus mirabilis (3 ESBL), 4 Citrobacter freundii (2AmpC) and 1 *ESBL Serratia marcescens*. In AmpC producers there were only 2 resistant isolates within 10 nonsusceptible ones (mainly *E. cloacae*). In ESBL producers only one isolate was nonsusceptible and none resistant. Bacteria were isolated from respiratory specimens (19) followed by soft tissues (13) and faeces/rectum (7). Only 6 were from urine.

**Conclusion:** Temocillin was highly active in vitro against our ESBL and derepressed AmpC Enterobacteriaceae. Nonsusceptibility occurred mainly in AmpC *E. cloacae* and resistance in AmpC *E. cloacae* only. Temocillin remains an interesting alternative in treating multidrug-resistant Enterobacteriaceae infections in our setting.

### Trends in susceptibility of Gram-negative pathogens isolated from intra-abdominal infections in North America from 2003 to 2007 – the SMART Study

**R. Badal**, S. Bouchillon, D. Homan, A. Johnson, M. Hackel, J. Johnson, G. Bochicchio

(Schaumburg, Baltimore, US)

**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) program has been monitoring the activity of ertapenem, amikacin, cefepime, cefotixin, ceftazidime, ceftriaxone, ciprofloxacin, imipenem, levofloxacin, and piperacillin/tazobactam against Gram-negative bacteria isolated from intra-abdominal infections (IAI) since 2003. This report compares susceptibility levels in 2003 vs 2007 for key IAI pathogens in North America.

**Methods:** 7–13 labs in the U.S. collected 100 consecutive Gram-negative isolates from IAI each year. MICs were determined by broth microdilution following CLSI guidelines, and % susceptible (%) rates in 2003 and 2007 were compared for species with >20 strains per year. 2003/2007 n’s of *E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa* were 427/224, 147/75, 80/31, and 161/64, respectively.

**Results:** 30/36 drug/bug combinations analyzed showed decreased %S from 2003 to 2007, but only 4/36 (E. coli vs. ciprofloxacin and levofloxacin, *K. pneumoniae* vs ceftazidime, and *P. aeruginosa* vs imipenem) were statistically significant (p < 0.05). Average change and range of changes in %-S for all drugs for *E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa* were –3.1% (+1% to −9%), −5.1% (−1% to −8%), −0.4% (+6% to −11%), and −7.1% (−3% to −12%), respectively.

**Conclusions:** Although all species showed trends of decreasing susceptibility to most drugs, relatively small n’s precluded establishment of significance.
In all cases except ciprofloxacin and levofloxacin vs. *E. coli*, ceftazidime vs. *K. pneumoniae*, and imipenem vs. *P. aeruginosa*.

In vitro activity of ertapenem has not changed significantly against Gram-negative IAI pathogens in North America from 2003 to 2007.

**Methods:**
One-hundred and twenty five of quinolones resistant strains, is frequent, there being few alternatives for the appropriate concurrence of quinolone resistance, particularly in ESBL-producing Enterobacteriaceae. The frequency of extended-spectrum-

**Objectives:**
The Study for Monitoring Antimicrobial Resistance Trends (SMART) program has been monitoring the activity of ertapenem, amikacin, cefepime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, imipenem, levofloxacin, and piperacillin/tazobactam against Gram-negative bacteria from intra-abdominal infections (IAI) since 2003. This report compares 2003 to 2007 susceptibility levels for key IAI pathogens in Europe (EU).

**Methods:**
28–31 labs in EU collected 100 consecutive Gram-negative isolates from IAI each year from 2003 to 2007. MICs were determined by broth microdilution following EUCAST guidelines, and % susceptible (%S) rates in 2003 were compared to %S in 2007 for species with >30 strains per year. 2003/2007 %S of *E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, *E. aerogenes*, *C. freundii*, *M. morganii*, *P. mirabilis*, and *P. aeruginosa* were 101/1291, 189/256, 112/124, 125/166, 40/39, 55/92, 57/53, 90/129, and 200/223, respectively.

**Results:**
59/87 drug/bug combinations showed %S decreases (7 significant, p < 0.05) from 2003 to 2007; 20/87 increased %S (1 significant, p = 0.0235); 8/87 had no change. Average changes were −1.8% (*E. coli*), −2.7% (*K. pneumoniae*), +2.5% (*K. oxytoca*), −5.6% (*E. cloacae*), −0.9 (*E. aerogenes*), −4.4% (*C. freundii*), −5.2% (*M. morganii*), −4.1% (*P. mirabilis*), and −2.9% (*P. aeruginosa*). %S changes of ertapenem ranged from +1% (*E. coli*) to −9% (*C. freundii*).

**Conclusions:**
• Although most species showed a general decline in %S vs. study drugs, only 7 drug/bug combinations were statistically significant; 4 of those were for *E. coli*, against which ertapenem and imipenem remained 99% effective.
• SMART study drugs showed far less change in %S from 2003 to 2007 in EU than other regions of the world.

**In vitro susceptibility of quinolone-resistant *Escherichia coli* isolates with and without producing extended-spectrum β-lactamase to fosfomycin trometamol**


**Objectives:**
The frequency of extended-spectrum-β-lactamase (ESBL)-producing *E. coli* strains has been increased in Turkey, and all of these isolates are recovered from outpatients with uncomplicated Urinary Tract Infections (UTIs). It is common to find that the same plasmid coding for ESBL is also contains genes conferring resistance to several groups of antimicrobial agents, such as aminoglycosides and cotrimoxazole. The concurrence of quinolone resistance, particularly in ESBL-producing strains, is frequent, there being few alternatives for the appropriate oral treatment of uncomplicated UTIs caused by ESBL-producing microorganisms. The aim of this study was to evaluate and compare the efficacy of Fosfomycin trometamol (FMT) in the treatment of quinolone resistant *E. coli* with and without ESBL.

**Methods:**
One-hundred and twenty five of quinolones resistant *E. coli* strains isolated from urine of outpatients (68 strains) and hospitalised patients (57 strains) were screened for the presence of ESBL by double disk synergy test and the susceptibility of the strains to FMT was performed by disk diffusion methods. Clinical Laboratory Standard Institute (CLSI) criteria was considered in both methods.

**Results:**
Twenty-eight (41.2%) of outpatients and 35 (61.4%) of hospitalised patients isolates, a total of 63 (50.4%) strains were revealed ESBL. All of the ESBL negative isolates were sensitive to FMT. Only two ESBL producing *E. coli* strains (3.2%) isolated from urine of hospitalised patients were found to be resistant to FMT.

**Conclusion:**
The high efficacy of FMT against all tested ESBL positive and negative quinolone resistant *E. coli*, in addition to low side effects and pharmacokinetic properties, FMT could be a useful alternative for single-dose therapy of uncomplicated UTIs, especially in regions with high quinolone resistance *E. coli* infections.

**In vitro activity of fosfomycin against multidrug-resistant Enterobacteriaceae isolates from a Belgian hospital**

A. Jeurissen*, J.P. Ursi, J. Van Schaeren (Wilrijk, BE)

**Objectives:**
Fosfomycin, a phosphonic acid derivative, has a broad-spectrum activity against Gram-positive and Gram-negative bacteria. It inhibits an early step in bacterial cell wall synthesis and it has mainly been used in the treatment of urinary tract infections. In this study we aimed to evaluate the in vitro activity of fosfomycin against multi-drug resistant (MDR) Enterobacter spp. and against extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae.

**Methods:**
Between May 2007 and August 2008 all ESBL-producing *E. coli* (n = 22), ESBL-producing Klebsiella spp. (n = 12) and MDR Enterobacter spp. (n = 25) were collected consecutively from inpatients with a documented infection. Accepted collection sources were blood, respiratory tract samples, urine, and wound specimen. Bacterial identification and susceptibility testing was performed using Vitek 2 system (Vitek 2, Biorineirix, Durham, NC, USA). ESBL production was confirmed by a double disk test, as recommended by the Clinical and Laboratory Standards Institute (CLSI). MDR Enterobacter spp. were defined as those strains that were nonsusceptible to at least 3 out of 6 of the following antibiotics: temocillin, aminoglycosides, piperacillin/tazobactam, meropenem, cotrimoxazol, and quinolones. The minimal inhibitory concentration (MIC) of fosfomycin was determined by the E-test, according to the manufacturer’s instructions (AB Biodisk, Solna Sweden). Results were interpreted according to the CLSI for Enterobacteriaceae: susceptible ≤ 64 μg/ml, resistant ≥ 256 μg/ml. Quality control was performed by testing susceptibility of *E. coli* ATCC 25922 and Pseudomonas aeruginosa ATCC 27853.

**Results:**
The ESBL-producing *E. coli* strains exhibited the greatest susceptibility to fosfomycin. MIC range was 0.25 to 1024 μg/ml with MIC90 of 16 μg/ml. Subsequently, the ESBL-producing Klebsiella spp. strains had a MIC range of 1.5 to 96 μg/ml, with MIC90 of 16 μg/ml. The MDR Enterobacter spp. were the least susceptible to fosfomycin, having MICs distributions across a range of 0.19 to 1024 μg/ml, with MIC90 of 48 μg/ml and MIC90 of 64 μg/ml.

**Conclusion:**
Using the breakpoint of 64 μg/ml, 96% of the ESBL-producing *E. coli*, 92% of the ESBL-producing Klebsiella spp., and 88% of the MDR Enterobacter spp. would be regarded as susceptible. Our data suggest good in vitro activity of fosfomycin against MDR Enterobacteriaceae.
clavulanate. A difference of $\geq 5$ mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk is taken to be phenotypic confirmation of ESBL production. Isolates with a carbenicillin MIC of $\geq 1$ mg/ml were screened for carbenapenemases by the modified Hodge test and PCR for OXA-48, VIM, IMP and KPC genes.

**Results:** The in vitro activity of ertapenem was as follows (Table 1). The body sites of infections were bloodstream (34%), urinary tract (34%), skin and soft tissue (11%), lower respiratory tract (8%) and others (13%). MIC50 of ertapenem were lower than imipenem for all species, about two to five fold more active than imipenem. Despite this good activity, the MICs of ertapenem for ESBL-producing Enterobacteriaceae with reduced sensitivity to carbenapenem were higher than imipenem. Imipenem was active (MICs <2 mg/L) in 10 of 12 ertapenem non-susceptible isolates. No isolate was imipenem resistant and ertapenem susceptible. The modified Hodge test was positive in 9 of 23 isolates with reduced carbapenem sensitivity. Susceptibility to carbapenems most likely have ESBLs or AmpC enzymes plus impermeability and/or increased efflux.

**Conclusion:** The susceptibility of ertapenem was 1–2% less than imipenem in ESBL producing Enterobacteriaceae. Isolates with putative carbenapenemases were rarely encountered. The rest of the isolates with reduced sensitivity to carbenapenem mostly likely have ESBLs or AmpC enzymes plus impermeability and/or increased efflux.

**Table 1. In vitro activities of ertapenem and imipenem against Enterobacteriaceae in Turkey**

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>MIC50 (mg/L)</th>
<th>MIC90 (mg/L)</th>
<th>Range</th>
<th>Susceptibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> (ESBL+), n=166</td>
<td>0.064</td>
<td>0.25</td>
<td>0.012–24</td>
<td>99</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.064</td>
<td>0.25</td>
<td>0.012–24</td>
<td>99</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.19</td>
<td>0.25</td>
<td>0.125–1.5</td>
<td>100</td>
</tr>
<tr>
<td>Klebsiella spp. (ESBL+), n=162</td>
<td>0.094</td>
<td>0.38</td>
<td>0.008–32</td>
<td>97</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.094</td>
<td>0.38</td>
<td>0.008–32</td>
<td>97</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.19</td>
<td>0.25</td>
<td>0.125–32</td>
<td>99</td>
</tr>
<tr>
<td>Enterobacter spp., n=88</td>
<td>0.047</td>
<td>0.5</td>
<td>0.008–32</td>
<td>97</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.047</td>
<td>0.5</td>
<td>0.008–32</td>
<td>97</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.25</td>
<td>0.38</td>
<td>0.125–32</td>
<td>99</td>
</tr>
</tbody>
</table>

**Conclusion:** Multidrug resistant bacteria showed resistance even to colistin, although this is a rare phenomenon. Klebsiella pneumoniae displayed the highest rates of resistance. There was no statistically significant difference in resistance across periods. Colistin may be effective for infections caused by multidrug resistant Gram negative bacteria, but precautions should be taken to restrict unnecessary usage in order to avoid increased resistance in the future.

**P1041 Molecular mechanisms of resistance to rifaximin in “in vitro” selected entero-toxigenic and enter-aggregative *Escherichia coli* mutants**

M.J. Pons*, L. Mensa, J. Vila, J. Gascón, J. Ruiz (Barcelona, ES)

**Objective:** The main aim of this study was to analyse the role of efflux pump and point mutations in the rpoB gene in the development of rifaximin (Rx) resistance in *Escherichia coli*. 

**Methods:** Bacterial strains: Parental *E. coli* (2 entero-toxigenic and 2 enter-aggregative) were isolated from patients with traveller’s diarrhoea in the Hospital Clinic of Barcelona. Fifteen Rx resistant mutants were selected after growing four *E. coli* clinical isolates in media containing Rfx.

Efflux pump: Susceptibility to Rfx was determined by agar dilution method, both in absence and presence of Phe-Arg-beta-naphthylamide (PAbetaN), an efflux pump inhibitor.

**Mutations in the rpoB gene were determined by PCR amplification of fragment of 848 pb, using the following primers and conditions: 5′-AAG CTC ATC GAT ATC CGT AAC G3′ and 5′-GCT TAT CAG CAC GCA GAG TCG GAA-3′, 30 cycles to 94°C during 30′, 60°C during 30′ and 72°C during 30′, final elongation 72°C during 10′). The PCR product was recovered using a commercial kit and sequenced.

**Results:** The results obtained show that the two analyzed mechanisms are implicated in the Rfx resistance. Amino acid substitutions at positions 516 and 526 of the beta-subunit of RNA polymerases, previously described in rifampicin resistance, were the most frequently obtained. Additionally new undescribed point mutations (512, 525 and 574) were found, although the results suggest that some of them (512, 525) do not play a relevant role (Table).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Initial MIC</th>
<th>Inhibitor effect</th>
<th>Amino acids position change</th>
</tr>
</thead>
<tbody>
<tr>
<td>23233 parental</td>
<td>16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>19769 parental</td>
<td>16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>21835 parental</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>19768–32*</td>
<td>128</td>
<td>8×</td>
<td>–</td>
</tr>
<tr>
<td>19766–64*</td>
<td>&gt;128</td>
<td>≥16×</td>
<td>526</td>
</tr>
<tr>
<td>19768–128*</td>
<td>&gt;128</td>
<td>≥16×</td>
<td>516</td>
</tr>
<tr>
<td>19769–16*</td>
<td>&gt;128</td>
<td>≥16×</td>
<td>525</td>
</tr>
<tr>
<td>19769–64*</td>
<td>&gt;128</td>
<td>≥16×</td>
<td>525</td>
</tr>
<tr>
<td>19769–32*</td>
<td>&gt;128</td>
<td>≥16×</td>
<td>525</td>
</tr>
<tr>
<td>19768–128*</td>
<td>&gt;128</td>
<td>≥16×</td>
<td>525</td>
</tr>
<tr>
<td>21835–16*</td>
<td>&gt;128</td>
<td>≥16×</td>
<td>512</td>
</tr>
<tr>
<td>21835–32*</td>
<td>&gt;128</td>
<td>≥4×</td>
<td>574</td>
</tr>
<tr>
<td>21835–64*</td>
<td>&gt;128</td>
<td>≥4×</td>
<td>574</td>
</tr>
<tr>
<td>21835–128*</td>
<td>&gt;128</td>
<td>≥4×</td>
<td>516</td>
</tr>
<tr>
<td>23233–16*</td>
<td>&gt;128</td>
<td>≥4×</td>
<td>526</td>
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<td>516</td>
</tr>
<tr>
<td>23233–128*</td>
<td>&gt;128</td>
<td>≥4×</td>
<td>516</td>
</tr>
</tbody>
</table>

*Antimicrobial concentration in which strains were isolated.

Except in four strains, PAbetaN presented an inhibitory effect, decreasing the MIC of rfx from 4 to 16 fold. However, this decrease in the Rfx
MIC could not explain the total resistance levels (because parental MIC was lower). In 6 mutants the efflux pump was able to explain the total resistance in 4 mutants only were observed mutations in the rpoB gene, while a the remaining mutants the resistance would be explained by the addition of two mechanisms.

**Conclusions**: All mutants strains presents at least one mechanisms of RfX resistance, both mutations studied in the rpoB gene and PAβETN inhibible efflux pump contribute for the RfX resistance (together or not). The possible role of others mechanisms of resistance (such as alterations in the expression levels of porins) may not be ruled out.

**Results**: Resistance data were analysed by using the WHONET 5.4 computer program. The standard agar dilution method according to the CLSI guidelines. The isolates to ciprofloxacin, nalidixic acid and azithromycin were tested by collecting from travellers returning from Southeast Asia. The MICs of the isolates to ciprofloxacin, nalidixic acid and azithromycin were tested by the standard agar dilution method according to the CLSI guidelines. The susceptibility data were analysed by using the WHONET 5.4 computer program.

**Methods**: 599 S. enterica isolates collected from Finnish travellers returning from abroad between 2003 and 2008 were enrolled in this study. 46 of these isolates belonged to the qnr-phenotype and were collected from travellers returning from Southeast Asia. The MICs of the isolates to ciprofloxacin, nalidixic acid and azithromycin were tested by the standard agar dilution method according to the CLSI guidelines. The susceptibility data were analysed by using the WHONET 5.4 computer program.

**Results**: Azithromycin resistance (MIC $\geq 32$ μg/ml) detected in 17 (2.8%) and high-level resistance (MIC $\geq 128$ μg/ml) in 8 (1.3%) of the collected isolates. Among the qnr-phenotype, azithromycin resistance was detected in 6 out of 46 (13.0%) isolates and high-level resistance in 1 (2.2%) isolate. Those azithromycin resistant isolates were collected between 2003 and 2004 and they belonged to the S. Stanley serovar. In the whole population, azithromycin resistance was the most abundant (5 isolates) in 2003 and 2004; thereafter, resistance decreased temporarily but has now increased again. Azithromycin resistance was detected in different serovars, S. Stanley being the most common one.

**Conclusion**: We show in this study that azithromycin resistance is uncommon among the whole Salmonella population. Resistance is uncommon also among the qnr carrying isolates. Moreover, azithromycin resistance has not been detected during the last four years. Thus, azithromycin might be a good treatment alternative for patients with reduced fluoroquinolone resistant isolates although highly azithromycin resistant isolates do occur.

**Molecular biology – part 1**

**P1045 In vitro activity of azithromycin against qnr carrying Salmonella enterica**

M. Lindgren*, P. Kottulainen, P. Hoxininen, A. Sittonen, A.J. Hakonen (Turku, Helsinki, FI)

**Objectives**: Reduced fluoroquinolone susceptibility among salmonellas has increased worldwide. We have previously described a novel population of Salmonella enterica showing reduced susceptibility to ciprofloxacin (MIC $\geq 0.125$ μg/ml) but being susceptible or only low-level resistant to nalidixic acid (MIC $\leq 32$ μg/ml). Ciprofloxacin is a first-line drug to treat Salmonella infections. However, when infection is caused by reduced fluoroquinolone susceptible isolates, treatment failures may occur and therefore alternative antibiotics are needed. The aim of our study was to investigate whether azithromycin is effective against reduced fluoroquinolone susceptible isolates and qnr carrying isolates of S. enterica.

**Methods**: 599 S. enterica isolates collected from Finnish travellers returning from abroad between 2003 and 2008 were enrolled in this study. 46 of these isolates belonged to the qnr-phenotype and were collected from travellers returning from Southeast Asia. The MICs of the isolates to ciprofloxacin, nalidixic acid and azithromycin were tested by the standard agar dilution method according to the CLSI guidelines. The susceptibility data were analysed by using the WHONET 5.4 computer program.

**Results**: Azithromycin resistance (MIC $\geq 32$ μg/ml) was detected in 17 (2.8%) and high-level resistance (MIC $\geq 128$ μg/ml) in 8 (1.3%) of the collected isolates. Among the qnr-phenotype, azithromycin resistance was detected in 6 out of 46 (13.0%) isolates and high-level resistance in 1 (2.2%) isolate. Those azithromycin resistant isolates were collected between 2003 and 2004 and they belonged to the S. Stanley serovar. In the whole population, azithromycin resistance was the most abundant (5 isolates) in 2003 and 2004; thereafter, resistance decreased temporarily but has now increased again. Azithromycin resistance was detected in different serovars, S. Stanley being the most common one.

**Conclusion**: We show in this study that azithromycin resistance is uncommon among the whole Salmonella population. Resistance is uncommon also among the qnr carrying isolates. Moreover, azithromycin resistance has not been detected during the last four years. Thus, azithromycin might be a good treatment alternative for patients with reduced fluoroquinolone resistant isolates although highly azithromycin resistant isolates do occur.

**P1044 Clinical and analytical studies of a real-time PCR assay for the detection of toxigenic Clostridium difficile**

E. Ziegler, E. Tyler, S. Visser* (Waukesha, US)

**Objectives**: Clostridium difficile (C. diff) is a common cause of nosocomial diarrhoea and if left untreated can lead to complications such as colitis and toxic megacolon. The prevalence of C. diff infection (CDI) has been escalating, leading to increased patient care costs, morbidity, and mortality. The surge in infection rates and the emergence of a hypervirulent strain (NAP1) has underscored the need for a fast, specific, and sensitive alternative to currently available assays. A Real-Time PCR assay for the detection of C. difficile was developed and prospectively tested at three clinical sites.

**Methods**: Prodesse’s ProGastro Cd assay, targeting the toxin B gene (cdtB) consists of a three-step process including Stool Clarification, nucleic acid extraction on the NucliSENSe easyMAG, and Real-Time PCR on the Cepheid SmartCycler II. Analytical sensitivity was determined by testing serially diluted titered strains of C. diff spiked into liquid stool samples. Analytical specificity was assessed by testing a panel of microorganisms that cause similar disease states or are commonly present in stool. To evaluate clinical performance, a prospective multicentre clinical trial was performed comparing ProGastro Cd to the Tissue Culture Cytotoxin Neutralisation Assay (CTA). Disrepectant results were resolved via a combination of sequencing, culture, and EIA analyses.

**Results**: The ProGastro Cd Assay has a limit of detection of $1 \times 10^1$ CFU/ml of bacteria in clinical matrix and is able to detect from 5 to $5 \times 10^7$ copies/reaction of the positive control. None of the organisms included in the specificity panel were reactive with the assay. From sample preparation to result, the assay can be performed in as little as three hours. Pooled results from the clinical trial on 771 samples yielded a clinical sensitivity of 91.7% and a specificity of 94.7%. After discrepant analysis, the clinical sensitivity and specificity increased to 96.2% and 99.8%, respectively. There were no inhibited samples in the trial.

**Conclusions**: The ProGastro Cd assay is fast, sensitive and specific for detection of toxigenic C. diff. This assay provides results faster than CTA and more accurate than other available diagnostic tests and will be a useful assay to aid in the diagnosis of CDI.

**P1045 Real-time PCR assays for the detection of Clostridium difficile ribotype 017 and 027 strains**

M. Reijans*, B. Mulders, E. Kuiper, M. Rapnik, G. Simons (Maastricht, Leiden, NL; Martihor, SI)

**Objective**: Clostridium difficile is the causative agent of antibiotic-associated diarrhea and pseudomembranous colitis. Clinical and epidemiological data suggests that there is an increase in severity, frequency and relapse of C. difficile-associated infections (CDI) in Europe and North America due to hypervirulent C. difficile ribotype 027 strains. Recently, outbreaks due to the emerging PCR ribotype 017 have also been noticed. Real-time PCR assays for specific identification of these two new emerging ribotypes were developed. Each PCR ribotype specific probe was combined with an universal C. difficile probe in a duplex realtime PCR.

**Method**: A set of 34 C. difficile strains belonging to 9 different PCR ribotypes (ribotypes 001, 002, 012, 014, 017, 020, 027, 078, 126) which are frequently found in Europe, were screened by Amplified Fragment Length Polymorphism (AFLP). Ribotype specific AFLP fragments were excised and sequenced. BLAST analyses with these sequences were performed on the genome of C. difficile strain 630 and flanking primers were designed to amplify specific fragments of various PCR ribotypes. These PCR fragments were sequenced and compared with each other to identify ribotype specific polymorphisms. The specific polymorphisms for ribotype 017 and 027 were used to develop two realtime PCR assays in which the ribotype specific probes were combined with an universal C. difficile probe.

**Results**: The AFLP screening of 34 C. difficile strains resulted in the isolation and sequencing of 24 PCR ribotype specific AFLP fragments. In total, 43 ribotype specific polymorphisms were identified. Specific Molecular Beacon probes for PCR ribotype 017 and 027 were designed. In addition, a C. difficile universal Molecular Beacon probe was designed.
directed against the triose phosphate isomerase gene. Subsequently, the sensitivity and specificity of duplex realtime PCR assays combining a ribotype specific probe and a C. difficile universal probe were validated on 36 additional C. difficile strains and in agreement with the PCR ribotypes

Conclusions: We developed two duplex realtime PCR assays for the detection of emerging C. difficile ribotypes 017 and 027 strains combined with a C. difficile universal probe. The assays allows a rapid detection of C. difficile and specific identification of ribotypes 017 or 027.

**P1046** Optimisation and validation of an in-house-developed real-time PCR for detection of toxigenic *Clostridium difficile* strains in human faeces

D. Bakker*, I. Sanders, J. Corver, E. Kuijper (Leiden, NL)

**Background:** In most laboratories there is a need for a more rapid and sensitive diagnostic test to diagnose *Clostridium difficile* infection. Previously we have developed a real-time toxin B PCR (toxB PCR) with a sensitivity of 10^3-10^5 CFU/g faeces. The aim of this study was to increase the sensitivity and perform a retrospective study to validate the optimised toxB PCR.

**Methods:** To determine the sensitivity of the different combinations of pre-treatment techniques and DNA extraction methods, we spiked *C. difficile* in pooled *C. difficile* negative faeces in tenfold dilutions series from 10^3 to 10^7 CFU/g faeces. To increase the sensitivity of the toxB PCR we optimised primer, probe and magnesium chloride concentrations and tried different mastermixes. Ninety-four different reference *C. difficile* ribotypes were tested in the optimised toxB PCR to validate it. The retrospective study included 101 stored positive faecal samples (defined as enzyme-linked fluorescent immunoassay (ELFA) and cytotoxicity assay (n=40) or culture (n=61) positive) and 64 stored negative faeces samples (defined as ELFA and cytotoxicity assay (n=53) or culture (n=11) negative).

**Results:** The combination Stool Transport and Recovery buffer (Roche) pre-treatment and automatic DNA extraction (Roche) had the best sensitivity of 10^3 CFU/g faeces. The optimisation of the toxB PCR resulted in a new mastermix (Hotstar, QIAGen) and by new optimal concentrations of primer, probe and magnesium chloride.

All tested *C. difficile* PCR ribotypes were detected by the optimised toxB PCR. The retrospective study revealed that all 40 ELFA/cytotoxicity positive faecal samples were detected. Six ELFA/cytotoxicity negative faecal samples were positive in the toxB PCR. All culture negative faecal samples were negative in the optimised toxB PCR. The optimised toxB PCR detected 59 of the culture positive faecal samples. Discrepancies in the retrospective study were analysed by reculturing faeces samples and retesting DNA samples by toxB PCR. We were able to culture toxigenic *C. difficile* in 5 ELFA/cytotoxicity negative/toxB positive samples. One culture positive/toxB negative was confirmed positive.

**Conclusion:** All tested PCR ribotypes were detected by the optimised toxB PCR. The optimised toxB PCR has a comparable sensitivity as the culturing method. Using culturing and ELFA/cytotoxicity as standard the sensitivity, specificity, PPV and the NPV of the modified PCR was respectively 98%, 91%, 94% and 96%.

**P1047** Evaluation of commercial toxin detection and two nucleic acid amplification tests for the routine detection of *Clostridium difficile* infection

S. d’Arc*, C. Thomas, K. Bamford (London, UK)

**Objective:** Identification of toxigenic *C. difficile* by cell cytotoxicity assay (CCAT) (gold standard) is only 70–80% as sensitive compared with culture. Enzyme immunoassay (EIA) (sensitivity ≥90% versus CCAT) may be less sensitive compared to culture. We aimed to compare the sensitivity and specificity of the Premier tox A & B, and two nucleic acid amplification (NAAT) platforms, Xpert™ and PROGASTRO™ in detecting toxigenic *C. difficile* with culture. In addition the NAATs were assessed for their ability to make a presumptive identification of the highly pathogenic ribotype 027 strain.

**Methods:** 147 stool samples submitted to the diagnostic laboratories at Hammermith Hospital for *C. difficile* testing were studied. Culture was performed using alcohol shock followed by isolation on *C. difficile* selective media (Oxoid). All tests were performed according to the manufacturer’s instructions. The PROGASTRO™ extraction was carried out using the QIAamp DNA stool mini kit (QIAGEN) and realtime PCR was performed on a RotorGene 6000 (n=92). Ribotyping was carried out according to published guidelines.

**Results:** *C. difficile* was isolated from 19 of the 147 samples, 18/19 were toxigenic: toxin B gene (tcdB) positive by NAAT or toxin A or B positive in EIA. The Xpert™ system was also able to detect the gene for binary toxin (cdt) and a deletion at position 117 of the regulatory gene TcdC, which if both are present alongside toxin B provides a presumptive identification of ribotype 027. Of the confirmed Xpert™ positives 1/19 (6%) was a presumptive ribotype 027, this was confirmed by Ribotyping. 22% were binary and toxin B positive, a feature of ribotype 078 and 72% only toxin B positive. Table 1 summarises the results of each assay. Of the toxin detecting assays the EIA had lowest sensitivity (36.8%) but highest specificity (99.2%). The Xpert™ provided the highest sensitivity of 94.7% with a specificity of 95.3%.

<table>
<thead>
<tr>
<th>Assay Result Comparison to culture positive + toxin positive results*</th>
<th>Assay</th>
<th>Result</th>
<th>Comparison to culture positive + toxin positive results</th>
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<td></td>
<td>Negative</td>
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<td>76</td>
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*a Pos = positive, Neg = negative, Sens = sensitivity, Spec = specificity, PPV = positive predictive value, NPV = negative predictive value.

**Conclusion:** Both NAAT methods produced sensitivity rates significantly higher than the EIA assay but in terms of ease of use and turnaround time the Xpert™ was superior. The Xpert™ is also able to differentiate ribotype 027 from non 027 strains, while the PROGASTRO™ only detects toxin B. Based on this work NAATs should be used for detection of toxigenic *C. difficile* in routine clinical samples. The addition of a method for rapid identification of type 027 is useful.

**P1048** A MLA-oligochromatographic multiplex assay for identification of the toxin genotype and the hypervirulent ribotype 027 of *Clostridium difficile*

B. Duremne, T. Laurent, T. Leclipteux, M. Rejuns, G. Simons, E. Kuijper, M. Runnik, P. Mertens* (Gembloux, BE; Maastricht, Leiden, NL; Maribor, SI)

**Objective:** *Clostridium difficile* is responsible for intestinal infections after disturbance of normal gut flora. Enterotoxin A (TcdA) and cytotoxin B (TcdB) are the main virulence factors but binary toxin CDT could also play a role in pathogenicity. Besides sporadic infections, *C. difficile* is responsible of nosocomial antibiotic-associated diarrhoea, a major epidemiological concern, particularly since recent outbreaks due to hypervirulent NAP1/027 type. Fast identification of toxigenic strain and ribotype 027 is crucial for both patient care and disease control. Multiplex ligation-dependent probe amplification (MLPA) that allows multiple target identification in one reaction and oligochromatography
(OC) that offers a fast and efficient detection of MLPA product are ideal for developing such an assay. We describe here a MLPA-OC assay applied on C. difficile toxin genes and ribotype 027 identification.

**Method:** Probes targeting different toxin genes as well as 027 specific probes were recently identified and adapted in a MLPA assay. Oligochromatographic strips were designed with 6 immobilised probes capturing specifically each MLPA product (tcdA, tcdB, cdtA, cdtB, 027 and tpi as marker of C. difficile) and II) a colloidal gold-probe conjugate, hybridising with all MLPA products, and allowing the detection with naked eye.

**Results:** Proof-of-principle of MLPA-OC was assessed using genomic DNA extracted from culture of C. difficile of 6 different ribotypes including 027. Culture supernatant (24h and 48h) of 5 strains were also tested successfully even without DNA extraction step. No cross-reactivity was observed with DNA extracted from negative fecal sample. DNA extracted from human fecal sample spiked with genomic DNA of C. difficile 027 shows that human DNA and/or stool specimen have an inhibitory effect on MLPA-OC. The total procedure (from fecal sample extraction to result) takes only 4h30 but overnight hybridisation during MLPA step increases the intensity of the signal.

**Conclusions:** We developed a MLPA-OC targeting the toxin genes of C. difficile (tcdA, tcdB, cdtA, cdtB) in addition to a 027 specific marker. The multiplex testing from the test relies on its easiness: one assay to detect 6 targets and only one cycler and a heating block are necessary for the entire procedure. The use of MLPA-OC as a diagnostic tool will require further optimisation of the extraction procedure, an improvement of the analytical sensitivity and a shorter protocol.

**P1049 Evaluation of a rapid molecular-based method, BD GeneOhm Cdiff Assay™ for the detection of toxin-producing C. difficile in fecal specimens**

G. Terhes*, E. Urbán, E. Nacsu, E. Nagy (Szeged, HU)

**Objectives:** Since 2002, the emergence and epidemics of hypervirulent C. difficile PCR ribotype 027, the raising morbidity and mortality rates, and the increasing number of recurrences and therapeutic failures have highlighted the necessity of a rapid and reliable method for the detection of toxin-positive strains in fecal specimens. The BD GeneOhm Cdiff Assay™ is a qualitative RT-PCR performed on the Cepheid SmartCycler® (Cepheid, Sunnyvale, CA) for the detection of tcdB gene. Our aim was to compare the results given by BD GeneOhm Cdiff Assay™ to the result of cytotoxicity tissue culture assay and a commercially available ELFA test for the direct detection of toxin A and B from fecal supernatant and to make an accurate assessment of the cost/benefit of this molecular method.

**Methods:** During two-month period 447 liquid to soft stool specimens were collected from both inpatients and outpatients in various departments of Szent-Györgyi Albert Medical Center (Szeged, Hungary). These samples were tested with VIDAS® Toxin A and B (BioMerieux, France) and cytotoxicity assay, and simultaneously BD GeneOhm Cdiff Assay™ was also set up in every case. The stool samples were plated on the surface of Clostridium difficile agar base (Oxoid, Basingstoke, UK) for culture.

**Results:** 8.3% of the examined 447 samples proved to be positive by BD GeneOhm Cdiff Assay™, while the prevalence of positive samples was only 6.7% and 5.1% using cytotoxicity assay and VIDAS® Toxin A and B (BioMerieux, France), respectively. 5.4% of the tested samples were positive with both molecular method and cytotoxicity assay. Testing consecutive samples from three patients showed that the presence of toxin-producing strain could be detected earlier using molecular method, when the toxin titre was under the detection level.

**Conclusions:** The laboratory standard to set up accurate diagnosis of CDAD is the simultaneous use of cytotoxicity assay or ELISA for toxin detection and selective culture of C. difficile, which is enough labour intensive and time consuming, mainly if the toxin detection directly from stool sample is negative and the culture is positive; repeated testing on the presence of toxins from broth culture is necessary to clarify the toxigenic status of the isolated strain. BD GeneOhm Cdiff Assay™ provides more reliable results than other toxin detection tests during a short period of time, which is very important from therapeutic and infection control points of view.
real time, PCR quantitative & Immunohistochemical) was made. A total of 256 clinical specimens were tested (whole blood, plasma, biopsy, cerebrospinal fluid, swab) being a true positive result judged according to the concordance between both methods. All the discrepancies were validated with sequencing, homemade PCR and nested-PCR.

**Results:** 100% analytical sensitivity was obtained in the detection of 14 recombinant plasmids between 10 and 1000 copies. It was observed that there were no cases of unspecific detection of viruses, which mean a 100% analytical specificity. About the diagnostic sensitivity and specificity, the behaviour of each virus after the validation of 256 clinical specimens showed that most of viruses have sensitivity higher than 90%, and specificity higher than 97%.

**Conclusion:** CLART® Entherpex is useful in the clinical setting for rapid screening and detection of a Human Herpesviruses and Enteroviruses based on the following facts: (i) excellent specificities and sensitivities; (ii) rapid and automatic procedure; (iii) simultaneous detection allowing the recognition of co-infections.

**P1052** Spectrum of pathogens in surgically treated infective endocarditis patients
T. Freiberger, E. Nemcová*, B. Zaloudišková, B. Krulova, J. Pol, Z. Sorm, M. Kolek, K. Noctova, P. Nemec (Brno, Hradec Králové, Ostrava, Prague, CZ)

**Objectives:** To characterise a spectrum of pathogens in Czech patients with infective endocarditis (IE) requiring surgical treatment.

**Methods:** Pathogens were detected in tissue heart valve samples from 106 IE patients surgically treated in four centres in the Czech Republic from 1.1.2006 to 31.12.2008, using 16S rRNA broad-range PCR followed by direct sequencing. The results were available within 24 hours in a routine clinical setting.

**Results:** Staphylococcus spp. were found in 51 cases (48.1%), Streptococcus spp. in 33 cases (31.1%) and Enterococcus faecalis in 7 cases (6.6%). Staphylococci were represented by S. aureus in 35 and coagulase-negative staphylococci in 16 cases, streptococci by S. pyogenes (1), S. agalactiae (5), S. anginosus group (8), S. mitis (4), S. mutans (2), S. sanguinis (2), S. galactosidus (2), S. infantarius (1), S. suis (1), S. cristas (1) and non-specified streptococci (6). Remaining cases included Bartonella quintana (2), Bartonella sp. (2), Cardiobacterium sp. (2), Haemophilus sp. (1), Gemella haemolytica (1), Lactococcus garvieae (1), Corynebacterium simulans (1), Propionibacterium acnes (1), T. whipplei (1), Proteus sp. (1), Pseudomonas aeruginosa (1), and Enterobacteriaceae (1).

**Conclusion:** Staphylococcus spp., Streptococcus spp. and Enterococcus faecalis were responsible for vast majority of IE cases requiring surgical treatment (85.8%). Broad-range 16S rRNA PCR followed by direct sequencing was shown as a power, rapid and very useful tool for identification of causative agents of IE from tissue samples to the species level, including differentiation of streptococci and detection of rare pathogens.

**P1053** Detection of OXA carbapenemase production in clinical samples by the Hyplex® CarboXa ID multiplex PCR-ELISA system
E. Kranotaki, E. Platsouka*, E. Pericoliotti, M. Nekpa, Z. Psaroudaki, O. Paniara (Athens, GR)

**Objective:** During the last decade, nosocomial outbreaks of multidrug-resistant strains of Acinetobacter baumannii, especially among immuno-compromised patients in intensive care units (ICUs), have been reported in diverse geographical areas. Moreover, the recent development of Actinetobacter strains producing the class D carbapenemases (mainly oxacillinases), is of great concern for the international community, since carbapenemases were often used to treat infections caused by multiresistant A. baumannii isolates. Hyplex® CarboXa ID multiplex PCR-ELISA (BAG Health Care, Germany) is a new diagnostic method for the direct detection of OXA-carbapenemases genes in clinical specimens.

**Methods:** The hyplex®CarboXa ID method involves amplification of genes for the identification of A. baumannii and the blaOXA-23, blaOXA-40 and blaOXA-58 gene families, by multiplex PCR and hybridisation of the PCR products to specific oligonucleotide probes in an ELISA-based system. The method was tested in 105 samples from different patients (30 positive blood cultures, 30 urine samples, 8 pus swabs and 37 bronchial secretions), obtained during September 2007 in Evangelismos, Athens hospital. Results were compared to those of a blaOXA-PCR screening of the tested isolates.

**Results:** Twenty-seven out of the 105 samples (20 bronchial secretions and 7 blood cultures) were positive by the hyplex®CarboXa ID. In details, all 27 samples were positive with the A. baumannii specific and the blaOXA-58 family specific probes. The A. baumannii isolates were impenetrant and meropenem resistant. By PCR, blaOXA-58 carrying A. baumannii isolates were detected from all the above hyplex-positive samples. None of the blaOXA-58 carrying A. baumannii isolates were identified in any of the hyplex-negative samples. Sensitivity and
Identification of TEM-type extended-spectrum β-lactamases genes based on real-time PCR and pyrosequencing

T. Naas*, N. Merad, P. Nordmann (Le Kremlin-Bicêtre, FR)

Objectives: Extended-spectrum β-lactamases (ESBLs) are increasingly prevalent worldwide being mostly CTX-M, TEM and SHV enzymes. Positive PCR results are sufficient to indicate the presence of a CTX-M-type ESBL, whereas sequencing is required for TEM-enzymes, since fast broad-spectrum (TEM-1/2) enzymes are highly prevalent. Therefore, a fast real-time PCR amplification, using TEM-specific primers coupled to real-time pyrosequencing was developed.

Method: A fast real-time PCR amplification based on a LightCycler 2.0 amplification system (Roche Diagnostic), using primers specific for the blaTEM alleles coupled to pyrosequencing (Biotage) was developed. Ten well-characterised TEM producers, representing various TEM alleles (TEM-1, different TEM-ESBLs and combinations of TEM-1/TEM-ESBLs) were used as controls. This high throughput technique has been evaluated by screening 20 ESBL producing E. coli isolates recovered from the Bicêtre hospital (France) in 2007. Bacterial DNA was extracted by boiling and using QiAmp Viral RNA extraction kit (Qiagen).

Results: The real-time PCR method detected 12/38 E. coli producing AmpC of the CIT subtype. Amongst the P. mirabilis isolates 4/5 were producers of the CIT subtype. Sequence analysis is ongoing to determine the exact genotypes, as well as epidemiological typing and plasmid replicon typing. The novel assay was less laborious compared to the conventional multiplex-PCR protocol currently in use. The PCR reaction was also faster, the run time being less than two hours including detection.

Conclusion: The real-time PCR method for pAmpC detection is a fast and simple screening method that gives information on the presence, as well as subtype of pAmpC. The SYBRGreen detection assay is relatively inexpensive, making it feasible also in a low income setting. The epidemiology of pAmpC in Sweden will be studied during 2009 using this methodology.

Multiplex real-time PCR detection of plasmid-mediated AmpC


Objectives: Plasmid-mediated AmpC (pAmpC) confer resistance to penicillins, cephamycins, oximino-cephalosporins and monobactams, and are currently being detected in Escherichia coli, Klebsiella spp., Salmonella spp. and Proteus mirabilis. E. coli has a chromosomal AmpC which can be hyper-produced, making it phenotypically difficult to discern from plasmid-mediated AmpC. Infection with AmpC producing bacteria is of great concern since treatment options are limited, and detection of such enzymes is consequently of epidemiological importance. All AmpC enzymes are inhibited by clavulanic acid and can thus be detected by synergy disc diffusion test. Phenotypic tests specific for pAmpC are however lacking. Detection of pAmpC therefore requires genetic methods such as PCR. β-lactamases of the pAmpC type can be divided into six subgroups based on nucleic acid identity; CIT, MOX, FOX, ACC, DHA and EBC.

Methods: We optimised a currently available conventional multiplex PCR to a real-time SYBRGreen PCR method. New primers for the ACC subgroup were designed to include detection of more recently described variants. The six primer pairs were divided in two triplex reactions, and PCR-products were separated through melting point analysis. The same temperature profile was used for both reactions, enabling common cycling.

A collection of 43 phenotypically determined (cloxacillin synergy test) AmpC producing E. coli (n=38) and P. mirabilis (n=5) from Skåne County, Sweden, was studied for the presence of pAmpC. Genes detected in the PCR were analysed by sequence analysis for determination of the exact genotype.

Results: The real-time PCR method detected 12/38 E. coli producing AmpC of the CIT subtype. Amongst the P. mirabilis isolates 4/5 were producers of the CIT subtype. Sequence analysis is ongoing to determine the exact genotypes, as well as epidemiological typing and plasmid replicon typing. The novel assay was less laborious compared to the conventional multiplex-PCR protocol currently in use. The PCR reaction was also faster, the run time being less than two hours including detection.

Conclusion: The real-time PCR method for pAmpC detection is a fast and simple screening method that gives information on the presence, as well as subtype of pAmpC. The SYBRGreen detection assay is relatively inexpensive, making it feasible also in a low income setting. The epidemiology of pAmpC in Sweden will be studied during 2009 using this methodology.

RAPID RAPID rapid detection and identification of zygomycetes in clinical samples using high-resolution melt analysis

K. Hrnčířová*, M. Lengerová, I. Kocmanová, Z. Racić, B. Weinbergerova, P. Volfova, D. Dvorakova, S. Pospisilova, J. Mayer (Brno, CZ)

Objectives: Invasive fungal infections are life-threatening complication in haematological/oncological patients. Recent studies have shown an increasing incidence of rare fungal infections as zygomycoses. For successful treatment rapid and accurate diagnostic methods are needed and molecular diagnostics is very helpful.

Methods: DNA from clinical samples and zygomycete strains was isolated with ZR Fungal/Bacterial DNA Kit (Zymo Research), from tissue samples with DNeasy Blood & Tissue Kit (Qiagen). For DNA detection we adopted previously published qualitative semi-nested PCR method for detection of Zygomyces targeting 18S region of ribosomal DNA. We optimised it for use as semi-nested real-time PCR with EvaGreen intercalation dye followed by species identification using High Resolution Melt (HRM) analysis on Rotorgene 6000 (Cobett Research).

Figure 1. High Resolution Melt (HRM) analysis. +C: positive control.
Results: We tested 83 samples from 53 haematological-oncological patients in risk of fungal infection: infected tissue (n=7), sputum (n=4), BAL (n=49), peripheral blood (n=19), 16 were taken concurrently with BAL samples and positive culture isolates (n=4), 7 tissue samples, 1 sputum sample, 16 BAL samples, 2 blood samples and all culture isolates were PCR positive. In 17 samples we were able to directly determine zygomycete species (Absidia spp. (n=4), Rhizomucor spp. (n=5), Rhizopus oryzae (n=4) and Rhizopus microsporus (n=4)) using HRM analysis and comparison with HRM curves of zygomycete reference strains, other 13 samples need to be sequenced to clarify obtained data.

Conclusion: Presented assay enables inexpensive, fast and reliable detection of zygomycetes in clinical samples (see Figure 1). HRM analysis of PCR products amplified with zygomycetes-specific primers allows determining of a broad spectrum of zygomycetes species (e.g. Rhizopus spp., Mucor spp., Rhizomucor spp., Cunninghamamella spp. and Absidia spp.) in one reaction. Compared to qualitative semi-nested PCR it does not require gel electrophoresis and time consuming sequencing of all PCR positive samples.

**P1058** Genotypic analysis of human *Echinococcus granulosus* strains in Turkey


Objective: *Echinococcus granulosus* is the causative agent of hydatid cyst disease with considerable impacts on human/animal health and economy. Although cystic hydatid disease continues to be endemic in our country, molecular epidemiologic data regarding the genotypes of *E. granulosus* strains infecting human beings are limited. The aim of this study was the molecular characterisation of *E. granulosus* strains obtained from human.

Methods: Between March-December 2008 cyst contents of 21 patients were collected during the to surgery in the surgery departments of different hospitals in Istanbul. The samples were checked for the absence of protozoa cephalically and were preserved in 70% ethanol at −20°C. Genomic DNA was extracted using a commercial DNA extraction kit (Nucleospin tissue kit, Macherey Nagel, Germany). A 446 bp. long part of mitochondrial c oxidase subunit 1 (CO1) gene of *Echinococcus granulosus* was amplified with PCR. PCR products were purified and sequenced in both directions with an automated DNA sequencer (ABI®, 310).

Results: Phylogenetic analysis showed that 16 of 21 human cysts belonged to the G1 genotype (common sheep strain) of *E. granulosus*. The sheep strain (G1 genotype) of *E. granulosus* was to be the predominant genotype present in humans in the study. This is the first molecular analysis performed on exclusively human strains of *E. granulosus* in our country.

Conclusion: According to the results of our study in our country, the *E. granulosus* strains of both human and animal origins belong to the same genotype.

**P1060** A real-time PCR for the detection of *Plasmodium ovale* variant strains

A. Calderaro*, G. Piccolo, C. Gorrini, S. Perazzi, C. Chezzi, G. Dettori (Parma, IT)

Objective: Malaria is the most frequent imported parasitic infection in Italy, mainly from Africa. Molecular assays based on 18S-rDNA developed by several researchers, including us, revealed that recently the prevalence of *P. ovale* (Po) infections was higher than previously thought and allowed to demonstrate that most prevalent malaria cases in our area as well as in Italy were due to *P. falciparum* (Pf), followed by *P. vivax* (Pv). Sequence variations in 18S-rDNA lead to describe a classic and a variant type of *P. vivax* (vtPo); therefore primers and probe specific for classic type of *Po* (ctPo) can fail in identification of vtPo. In this study, a new primer and probe for the identification of vtPo were described in order to accurately and promptly diagnose cases of imported malaria.

Methods: A new Rt-PCR assay to identify vtPo was developed using a new (OVA-Fv) and a previously described (OVA-R) primer with a new probe specific for vtPo (OVA-v) in a variant Rt-PCR (Rtv-PCR) assay. This assay was comparatively evaluated with the classic Rt-PCR assay (Rtc-PCR) to identify Po testing 24 selected blood samples from 24 patients with malaria. Some samples were also pre-amplified by a genus-specific conventional PCR (primers rPLU1-rPLU5) before testing with Rt-PCR assays.

Results: Among the 24 samples resulted positive for Po 15 were ctPo and 9 vtPo. Fourteen samples were Po positive by Rtc-PCR and 6 samples were Po positive by Rtv-PCR. Two samples resulted indeterminate by Rtc-PCR and Rtv-PCR, respectively, were positive by the same assays after pre-amplification. Two samples negative by both Rt-PCR assays resulted positive by Rtv-PCR after pre-amplification. No signal was detected by Rtv-PCR assay testing DNA from 4 Pf, 2 Pv and 5 Pm positive samples.

Conclusion: The present study reports that some cases of malaria by vtPo (9 of 24 in our experience) could not be diagnosed by both microscopy and Rtc-PCR based on 18S-rDNA. The use of Rtc- and Rtv-PCR to accurately identify Po strains can improve sensitivity of diagnostic assays, especially in case of malaria by Po, usually occurring with low parasitaemia as was particularly in 4 of 24 samples which needed a pre-amplification. The association of microscopic and molecular assays demonstrated to be essential for a rapid and accurate diagnosis of imported malaria in our area and allowed to administer...
Detection of aetiology of peritonitis in peritoneal dialysis patients by PCR

M. Suvorova*, A. Zemchenkov, P. Garosimchuk, V. Edelshtein, A. Sabodash, E. Tarasova (St. Petersburg, RU)

Introduction: Peritoneal dialysis becomes the method of choice for the patients with renal dysfunctions worldwide. This type of procedure allows people to live their ordinary life without staying in the hospital. The main setback of this procedure is an infection which causes peritonitis statistically once in 18 months (Kavanagh D.2004. According to the literature the most common bacterial pathogens causing peritonitis are: E. coli, S. aureus, S. epidermidis and C. albicans. Routinely in identification of the pathogen is done by bacteriological analysis with the following selection of antibiotics. However, quite often bacteriological method fails and in clinical practice wide spectrum antibiotics are used (Vancomycin, Amikacin) which is often ineffective and harmful for the normal microflora. The aim of present study was to identify the presence of bacterial pathogens in peritoneal fluids by PCR.

Materials and Methods: 20 patients with peritonitis after dialysis with negative bacteriological results have been selected for the study. Peritoneal fluid from the patients was used for DNA isolation with the following PCR. Material was primarily tested for the bacterial content using Universal primers for bacterial rRNA. Positive samples were studied by the specific primers to S. aureus S. pyogenes, S. agalactiae, E. faecium, E. faecalis, E. coli, S. pneumoniae, S. mutans, S. sobrinus, C. albicans. PCR negative samples were tested for the presence of C. trachomatis, Ureaplasma urealyticum, M. hominis and M. genitalium.

Results: After the preliminary study with universal primers 11 samples out of 20 were tested as positive for bacterial DNA. 9 samples were negative. Among the positive samples 6 contained the mixture of E. faecium and S. mutans. All the enterococcal strains carried sets of putative virulence genes efaA, asa1, gelF. 3 samples carried S. mutans strains with the putative virulence genes PA, PGP, PPGK. Only one sample carried S. aureus and one was positive for E. faecalis. In order to discover the cause of infection among the rest of the patients have been tested with DNA primers to Chlamydia and Mycoplasma. Three additional samples were tested positive with these primers.

Conclusions: So called aseptic cases of peritonitis are in reality quite often caused by bacterial infections missed by the classical cultural study. Chlamydia, Mycoplasma and S. mutans might be the cause of infection which is important for the proper choice antibiotic therapy.

Comparative analysis of serum proteomes to discover biomarker for scrub typhus

C.S. Lee*, H.N. Kim, Y.G. Kwak (Jeonju, KR)

Objective: Proteomics has the potential to identify noble biomarkers from pathologic tissue, biologic fluids and sera. In the present study, in order to find an easier and simpler diagnostic method and to find the pathogenic proteins of scrub typhus, the sera of patients were analyzed by proteomics techniques.

Methods: The 2-dimensional electrophoresis patterns of sera from acute febrile patients, pre-therapy and post-therapy of scrub typhus patients and normal subjects were compared. The differentially expressed spots were identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry.

Results: A total of 2,000 to 2,500 spots of protein of 130 g were observed when staining was done with routine silver staining procedures. The gender and age of the samples were matched as much as possible because human samples are rather notorious for their individual variations in the proteins’ expression level. We found twenty one proteins that were significantly and consistently different on 2-DE gels between the sera from the negative, pre- and post-therapy patients and the sera from the normal subjects. Thirteen proteins among them were up-regulated in the pre-therapy scrub typhus patients, whereas eight proteins were down-regulated. The results of the MALDI-TOF MS unequivocally indicated that the identities of the up-regulated spots were PR02044, PR02675, apolipoprotein E, albumin-like protein, glutathione peroxidase, WFDRC5, fibrinogen gamma, seren/cystein proteinase inhibitor clade G member 1 splice variant 2, complement factor B, alpha-1-B glycoprotein, the identities of the down-regulated spots were proapolipoprotein, immunoglobulin G1 Fe fragment, transferrin, Ig mu chain C region.

Conclusion: The proteins obtained with this proteomic analysis will be very useful in understanding the pathophysiology of scrub typhus. These proteins will also be useful in finding candidates as diagnostic biomarkers and new therapeutic targets for the treatment of scrub typhus.

Molecular profile of rotavirus in children hospitalised with acute gastroenteritis in Norway, 2006-2008


Objectives: Development of new rotavirus vaccines calls for the epidemiologic and molecular data on rotavirus diarrhoea to inform the policy decision on introduction of these vaccines into the national immunisation program. In order to explore the distribution of rotavirus strains in Norway, we conducted extensive strain surveillance among children <5 years of age hospitalised with acute gastroenteritis in three large hospitals during two consecutive rotavirus seasons in 2006-2008.

Methods: A stool sample was collected from each enrolled child during hospital stay. All samples were initially screened for rotavirus antigen by ELISA (DAKO Diagnostics). A subset of samples was also tested using a rapid immunochromatographic test (BioMerieux). Rotavirus G and P genotyping was performed using RT-PCR.

Results: Totally 311 stool samples were collected from enrolled children during the study period. Rotavirus was detected in 190 (63%) samples by ELISA and in 219 (72.3%) samples by RT-PCR. Only 2 (1%) ELISA-positive samples were RT-PCR negative. In a subset of 84 samples tested by both ELISA, rapid immunochromatographic test and RT-PCR, the rotavirus detection rates were 67%, 90% and 87%, respectively. Rotavirus G and P typing was performed on 219 RT-PCR-positive samples. The predominant G1 type was detected in 116 samples, accounting for 51% and 55% of strains during the first and second surveillance years, respectively. The second most frequently identified genotypes were G3 and G9, detected in 7.5% and 18.3% in 2006-2007 and 20.2% and 14.1% during 2007–2008, respectively. P[8] was identified in 188 samples (86%). The prevalence of common G and P combinations was >80% during the two consecutive surveillance years, and the G1P[8] genotype combination was identified in 49% of samples. No unusual rotavirus strains were detected, and only four samples contained mixed infections.

Conclusion: Rotaviruses responsible for severe gastroenteritis leading to hospitalisations in children <5 years in Norway include only globally common G and P genotypes. The observed geographical and seasonal variation in the distribution of rotavirus genotypes shows that further rotavirus strain surveillance in Norway is critical to monitor changes in circulating genotypes and determine how effective available vaccines may be in reducing severe rotavirus disease in Norwegian children.
mass peaks corresponding to ribosomal proteins. The aim of this study was to compare two commercially available MALDI-TOF MS systems in a multi-user setting of a routine microbiology laboratory. 

**Methods:** 245 bacterial and 34 yeast isolates were tested with: Microflex LT MALDI TOF System/Biotyper 2.0 SR1 software (Bruker Daltonics, D-Bremen) and Axima Assurance (Shimadzu, CH-Reinach) along with Saramis software (Anagnostec, D-Potsdam). Depending on the pathogen direct smear of colonies onto the target plate or extraction protocols were applied according to the manufacturer's instruction. Conventional identification was used as reference. It was performed with commercial systems, mainly Vitek 2 and API Systems (BioMérieux, F-Marcy l'Étoile).

For isolates with inconclusive biochemical identification or discrepant results sequencing of the 16SrRNA was performed.

**Results:** Correct identification for Axima/Saramis (AS) and Microflex/Biotyper (MB) at species level (S) or genus only level (G) were as follows:

- Enterobacteriaceae (n = 76): AS 62 (82%) S, 5 (7%) G; MB 59 (78%) S, 8 (11%) G
- Nonfermenters (n = 23): AS 17 (73%) S, 2 (9%) G; MB 13 (57%) S, 5 (22%) G
- Staphylococci (n = 16): AS 10 (63%) S, 2 (16%) G; MB 12 (75%) S, 2 (16%) G
- Enteroococci (n = 13): AS 9 (69%) S, 2 (15%) G; MB 11 (85%) S
- Streptococci (n = 32): AS 18 (56%) S, 5 (16%) G; MB 22 (69%) S
- Campylobacter jejuni coli (n = 23): AS 20 (87%) S, 1 (4%) G; MB 23 (100%) S
- Other bacteria (anaerobes, fastidious Gram neg, rods, Gram pos. rods) (n = 62): AS 17 (27%) S, 11 (18%) G; MB 28 (45%) S, 12 (19%) G
- Yeasts (n = 34): AS 29 (85%) S; MB 29 (85%) S, 1 (3%) G.

**Conclusions:** Both MALDI-TOF MS systems were rapid and accurate for the identification of the most frequently cultured bacteria and yeasts. Quality of results improved with time over the four weeks period of the evaluation. This shows that training skills are essential for sample preparation and particularly for applying the material on the target plates. In a clinical laboratory setting MALDI-TOF MS has the potential to replace conventional identification for the majority of bacteria and yeasts.

**Antimicrobial resistance in Gram-positive bacteria**

**P1066** Dermafinder: a new approach for fast and sensitive detection of dermatophyte skin infections


**Objective:** Superficial dermatophytosis is the most common fungal infection in humans. Dermatophytes are keratinophilic fungi which are able to infect keratinised tissue. Diagnosis of dermatophytosis is based on microscopic observation of fungal structures in KOH threated skin scales plus culturing and identification of the causative species. However, direct microscopy lacks specificity and culturing is slow because it requires generally 2–4 weeks. To address this we have developed a molecular test, the DermaFinder. The DermaFinder assay is able to detect the major 8 pathogenic dermatophytes in a single reaction.

**Method:** The DermaFinder is based on the MultiFinder technology which enables simultaneous amplification of up to 40 fragments. Primers and probes were designed based upon unique AFLP markers. The assay includes two probes targeting 2 dermatophytes species; Trichophyton rubrum combined with T. soudanense and Microsporum canis combined with Microsporum audouinii. The DermaFinder can detect four single dermatophytes: T. mentagrophytes, T. violaceum, T. tonsurans and Epidermophyton floccosum. In addition, the assay includes also one probe which detects all members of the Trichophyton genus.

**Results:** A set of skin samples (232) from sporters with athlete's foot were used to validate the DermaFinder assay. Results where compared with microscopy, KOH/blankophor and culture and showed a good correlation. A specific dermatophyte real-time assay was used to test the discrepancies. In most cases the real-time PCR confirmed the DermaFinder results. In total 38% (87) of the athlete's were suffering from a dermatophytosis. Moreover, the DermaFinder assay was able to detect an additional 25% (36) of pathogenic dermatophytes in culture negative samples.

**Conclusion:** The DermaFinder is able to detect the major 8 pathogenic dermatophytes in clinical specimens and proved to be more sensitive and specific than culture and direct microscopy. Earlier therapy and information on the source of infection is possible with this test.
drop and subsequent rebound in antimicrobial resistance, cubic splines were utilised in a logistic regression model. Multi-drug resistance was considered as full resistance to two or more antimicrobial classes.

Results: In children less than five years old, antimicrobial resistance continuously increased from 1996 to reach its peak between 2000 and 2001. 2001 rates were 48.8% for TMP/SMX, 44.9% for erythromycin, 32% for penicillin, 24.9% for tetracycline, 7.0% for ceftriaxone, and 29.5% for multiple resistance. All antimicrobial classes subsequently experienced a steep drop in resistance before leveling off between 2003 and 2004. Levels in 2004 were 35.3% for TMP/SMX, 38.1% for erythromycin, 21.1% for penicillin, 19% for tetracycline, 2.6% for ceftriaxone and 22.1% for multiple resistance. A rebound was also experienced in every class, with 2007 levels near or above the 2001 peaks. Resistance rates for 2007 were 41.1% for TMP/SMX, 45.7% for erythromycin, 27.6% for penicillin, 30.4% for tetracycline, 3.1% for ceftriaxone, and 27.9% for multiple resistance. Similar changes were observed by source of the isolate, with blood/CSF isolates having lower peaks and rebounds, and otitis media isolates showing very steep changes over time. Similar changes were also observed by inpatient/outpatient status and in different regions of the country, with larger variations among the regions that started with higher levels.

Conclusion: Most antimicrobial classes experienced a significant drop in resistance after the introduction of the pneumococcal vaccine in 2001. This effect reached its maximum in 2004 with a subsequent and significant rebound by 2007. The same pattern is seen regardless of specimen source, US census region or inpatient/outpatient status, and may be due to the fact that the vaccine serotypes were the most frequent and most resistant in 2001. As they were replaced by non vaccine serotypes, resistance declined initially but later increased as the non-vaccine serotypes acquired resistance.

Results: MIC50 and MIC90 of GEM and GRN were much lower than those of MXF and LVX. Efflux (as detected by R) was demonstrated (i) for GEM only (and for a small proportion of isolates) if using a 2-fold dilution increase criterion; (ii) for MXF, GRN and for GEM (affecting almost all isolates for GEM) if using a 1-fold dilution increase criterion. Conclusions: MICs of LVX in this collection support the “high dose” recommendation on which LVX EUCAST breakpoint is based (geom. mean more than 3-fold larger than what was reported in the study that established the usefulness of the 500 mg dose [0.25 mg/L; Preston et al., JAMA 1998;279:125–9]). MXF MICs remain under EUCAST breakpoint and similar to pre-marketing surveys in Germany (MIC90 = 0.25 mg/L [Reinert et al. JAC 1998;42:803–6]). Efflux is evident for GEM (in the absence of specific selection pressure) and likely for MXF and GRN (but with minor impact on MIC values).

Background: Belgium is a country with one of the largest fluoroquinolones use in Europe (2.36 DDD per 1,000 inhabitants and per day in ambulatory care, based on 2006 ESAC data [http://www.esac.ua.ac.be]). We have examined the MIC distribution and the influence of reserpine (efflux inhibitor) of MXF and LVX (in clinical use since >6 years) in comparison with GEM and GRN (not yet approved) towards S. pneumoniae (SP) isolated from patient with confirmed CAP.

Methods: 134 SP first isolates were collected over the 2004–2008 period from patients received from the community into hospital (6 institutions) and for whom clinical data were consistent with a diagnosis of CAP. MICs were determined by semi-geometric microdilution in CAMH broth + 2.5% lysed horse blood, with or without reserpine (R; 10 mg/L). Susceptibility was assessed according to EUCAST breakpoints for MXF and LVX.

Results: FQ MIC distribution and efflux of 4 respiratory fluoroquinolones (GEM, GRN, MXF, LVX) towards S. pneumoniae isolated from patients with confirmed CAP in a country with large fluoroquinolone use (Belgium)

<table>
<thead>
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<th>MIC distribution</th>
<th>EUCAST breakpoints</th>
<th>Efflux</th>
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<tr>
<td></td>
<td>geom. mean</td>
<td>MIC90</td>
<td>R</td>
</tr>
<tr>
<td>LVX</td>
<td>0.83</td>
<td>0.71</td>
<td>1</td>
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<tr>
<td>MXF</td>
<td>0.16</td>
<td>0.14</td>
<td>0.25</td>
</tr>
<tr>
<td>GRN</td>
<td>0.04</td>
<td>0.03</td>
<td>0.06</td>
</tr>
</tbody>
</table>

MIC distribution and efflux of 4 respiratory fluoroquinolones (GEM, GRN, MXF, LVX) towards S. pneumoniae isolated from patients with confirmed CAP in a country with large fluoroquinolone use (Belgium)

A. Lismond*, S. Carbonnelle, F. Van Bambeke, P. Tulkens (Brussels, BE)

Objective: Streptococcus pneumoniae remains a major cause of bacteremia and meningitis, and treatment may be complicated by non-susceptibility to penicillin. This study aims to identify trends in penicillin non-susceptibility rates in invasive S. pneumoniae in Scotland, and to determine if any serogroups/types are particularly associated with resistance.

Method: Data on invasive isolates of S. pneumoniae were obtained from Health Protection Scotland, and the Scottish Meningococcus and Pneumococcus Reference Laboratory. Antibiotic susceptibility data and serotypes for invasive isolates (identified from blood, CSF, or other normally sterile sites), between 1999 and 2007 were analysed.

Results: 4727 isolates were suitable for inclusion. Of these, 148 (3.13%), were of intermediate penicillin sensitivity (MIC = 0.12–1 mg/mL), and 7 (0.15%), were penicillin resistant, (MIC > 1 mg/mL). This is an increase from a previous Scottish study, which found 105 penicillin intermediate-susceptible isolates (1.85%), and only 2 penicillin-resistant isolates (0.04%), from the 5659 isolates collected between 1988 and 1999 (p = 0.00032). The highest recorded penicillin MIC was 16 mg/mL. Ten serogroups were identified among the non-susceptible isolates (1, 3, 6, 8, 9, 14, 19, 23, 35, 38), of which 5 serogroups (accounting for 14 (9.5%) of the non-susceptible isolates), are not covered by the 7-valent conjugate pneumococcal vaccine introduced into the UK in 2006. Seven of the non-susceptible isolates were used to cover the 7-valent conjugate pneumococcal vaccine introduced into the UK in 2006. Seven of the non-susceptible isolates was not covered by the vaccine (50%), were isolated in 2007 after the introduction of the vaccine. In total 4531 (95.8%) of the isolates were from blood, and of these, 143 (3.1%) showed reduced sensitivity to penicillin.

Conclusion: The rate of penicillin non-susceptibility amongst invasive S. pneumoniae isolates submitted between 1999 and 2007 has increased from 1.89% to 3.28% (p = 0.000004). Ten serogroups/types account for the penicillin non-susceptible isolates, and 5 of these are not covered by the conjugate pneumococcal vaccine currently used in the UK.
Regional variations in penicillin resistance rates against Streptococcus pneumoniae: The United States, 2008


Background: The percentage rates of penicillin-resistant (PenR) S. pneumoniae (SPN) vary by country and region. Earlier studies have documented U.S. regional variations in PenR SPN. The purpose of this study was to determine changes in regional variations, if any, of PenR and PenNS strains of SPN, and the current activity of tigecycline (TIG), amoxicillin-clavulanic acid (AC), ceftriaxone (CFX), levofloxacin (LEV), linezolid (LNZ) and vancomycin (VAN) to pen-resistant isolates.

Methods: 2,443 clinically relevant isolates of SPN were collected from patients in 193 hospitals from 2004-2008. MICs to all agents tested were determined by broth microdilution and interpreted following CLSI guidelines. Regions are defined by the CDC.

Results: PenNS rate was 42.4% for all regions varying from a high of 60.3% (East South Central) to a low of 32.3% (Pacific and New England). PenR decreased in all regions but one (New England) with a corresponding increase in PenN rates in most regions. Regional changes from a 1999-2000 study to 2004-2008 study are noted. Tigecycline and vancomycin had the lowest MIC90s (mg/mL) against PenR SPN at 0.5 followed by LEV and LNZ at 1 and CFX and AC at 2 and 8, respectively.

Conclusions: PenR rates for SPN remained essentially constant since 1999, but PenR has generally shifted from Pacific regions eastward. VAN, LNZ, LEV and TIG MIC90 values remain unaffected by penicillin resistance above the rates seen in 2007. Conversely, there appears to be decreased resistance to macrolides, tetracyclines and trimethoprim/sulfa.

Residence and resistance mechanisms in Streptococcus pneumoniae in the Netherlands the Duem3 study

J.W. Mouton*, H. de Valk, S. Meijers, C. Klaassen and the Duem Study Group

Objectives: In 1999, 2001–2, 2003 and 2005 we performed surveys in The Netherlands (NL) to monitor antimicrobial resistance in S. pneumoniae (SP). We then found increasing prevalence of macrolide resistance but virtually no resistance to quinolones. We here report the survey in 2007.

Methods: 26 laboratories equally distributed throughout NL participated in the survey. Each lab was asked to collect up to 25 pneumococcal strains of consecutive samples. Only blood or sputum (including lavage) was allowed. Identification was done by participating laboratories using their own standard identification technique; in any case bile solubility was required. MICs were determined using the Etest on site for Levofloxacin (Le), Moxifloxacin (Mo), Penicillin (Pe), Amoxicillin (Am), Clarithromycin (Cl), Cefotaxim (Ce), Cotrimoxazole (Ct) and Doxycyclin (Do); control ATCC strains were included. Data were entered using a web-based system. Afterwards, strains were collected by the central lab for further analysis. Identification confirmation of all SP was performed by bile solubility testing and also by LytA PCR for resistant strains. Resistance genes were identified using validated PCR-based methods for all strains with a MIC of >1mg/L for Le, and >0.5mg/mL for CL. EUCAST susceptibility criteria were used.

Results: 583 strains were tested, of which 542 were available and confirmed SP after reidentification. MIC90s in mg/L were 0.125 (Mo), 1 (Le); 0.023 (Pe), 0.047 (Ce), 1.5 (Cl), 0.38 (Do), 0.25 (St) and 0.023 (Am). Of the Cl resistant strains, slightly less than half were due to the erm type resistance and 41% due to efflux (mefA and mefE) showing a similar pattern as earlier surveys. 0.6% was LeR with a double mutation.

Conclusions: Over the years – during 5 surveys – macrolides showed a consistent and significant shift to higher MIC90 values. For the first time more than 10% of strains are now R for Clarithromycin, values that may prohibit blind prescribing of macrolides. In contrast, no significant increase in resistance was observed for β-lactams, quinolones, doxycyclin and cotrimoxazol.


K. Green*, A. McGeer, A. Pleasenti, S. Pong-Porter, D. Low on behalf of the Canadian Bacterial Surveillance Network (CBSN)

Objectives: To monitor trends in antimicrobial resistance in Canadian isolates of Streptococci pneumoniae (SPN). Methods: The Canadian Bacterial Surveillance Network (CBSN) is a collaborative network of microbiology laboratories from across Canada that has been monitoring resistance trends in Canadian isolates of SPN since 1993. Participating laboratories submit bacterial isolates to a central laboratory for standardised antimicrobial susceptibility testing performed according to CLSI protocols.

Results: Of the 31,064 SPN isolates submitted and tested between 1993 and July of 2008, 37% were from blood/CSF, 41% from sputum, 22% from other sites. The trends in antimicrobial susceptibility are expressed below as percentage resistant. Macrolide resistance and tetracycline resistance have both increased significantly between 2004 and 2008. Resistance to fluoroquinolones decreased slightly since 2006 (in 2007: Cip 1.7%; Levo 0.8%; Moxi 0.5%) but has increased slightly in all but moxifloxacin in 2008 (Cip 2.2%; Levo 1.6%; Moxi 0.7%). Penicillin resistance decreased in 2007 from to 6.2% in 2006 to 4.1%, P = 0.009, but appears to be increasing slightly again in 2008 (6%). Ceftriaxone resistance (meningal breakpoint MIC >2) increased to 3.3% in 2008 from 2.9%. Amoxicillin resistance continues to increase to 1.9% in 2008.

Conclusions: Preliminary data from 2008 suggest modest increases in resistance to penicillin, amoxicillin, ceftriaxone, ciprofloxacin and levofloxacin above the rates seen in 2007. Conversely, there appears to be decreased resistance to macrolides, tetracyclines and trimethoprim/sulfa.
by the local laboratory using supplied broth microdilution panels and interpreted according to EUCAST guidelines.

**Results:** Summary data for the 121 (26.3%) macrolide-resistant strains are presented in the table.

**Conclusions:** Tigecycline demonstrated the lowest MIC50 and MIC90 in vitro values of all study drugs against macrolide-resistant *S. pneumoniae*. Tigecycline in vitro activity suggests that tigecycline may be effective against this important clinical pathogen and resistant phenotype.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Macrolide-resistant <em>S. pneumoniae</em> (n=121)</th>
<th>MIC50</th>
<th>MIC90</th>
<th>% Susceptible</th>
<th>% Resistant</th>
</tr>
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<tbody>
<tr>
<td>Tigecycline</td>
<td>0.03</td>
<td>0.12</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>AmoxClav</td>
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<td>&gt;2</td>
<td>62.8</td>
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<td>100</td>
<td>0</td>
<td></td>
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<tr>
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<td>1</td>
<td>100</td>
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<tr>
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<td>62</td>
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na = breakpoints not defined.

**P1074** Macrolide resistance in *Streptococcus pneumoniae* in the United States and Canada, 2004–2008


**Background:** Even with decreases in antibiotic consumption in Canada, non-β-lactam resistant rates against *S. pneumoniae* are on the rise. The Tigecycline Evaluation Surveillance Trial (T.E.S.T) program is an ongoing global surveillance designed to follow trends in antimicrobial activity. This report evaluates tigecycline activity in United States and Canada against macrolide-resistant *S. pneumoniae* during the time from 2004 to 2008.

**Methods:** 1,256 clinical isolates were collected from 178 investigative sites in the United States and Canada. Clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to CLSI guidelines.

**Results:** Summary data for the 371 (29.5%) macrolide-resistant strains are presented in the table.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Macrolide-resistant <em>S. pneumoniae</em> (n=371)</th>
<th>MIC50</th>
<th>MIC90</th>
<th>% Susceptible</th>
<th>% Resistant</th>
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</tr>
</tbody>
</table>

na = breakpoints not defined.

**Conclusions:** Tigecycline demonstrated the lowest MIC50 and MIC90 in vitro values of all study drugs against macrolide-resistant *S. pneumoniae*. Tigecycline in vitro activity suggests that tigecycline may be effective against this important clinical pathogen and resistant phenotype.

**P1075** Trends of antimicrobial resistance among staphylococci and enterococci in Germany


**Objectives:** Tigecycline, which has been shown to have potent activity against a wide range of bacteria including multi-resistant pathogens such as MRSA and VRE, has been introduced in Germany in May 2006. The German Tigecycline Evaluation Surveillance Trial (G-TEST) is an ongoing surveillance programme comprising 15 laboratories monitoring the susceptibility of bacterial pathogens to tigecycline. The objective of this study was to compare the in vitro activities of tigecycline and other drugs against isolates of *S. aureus*, *S. epidermidis*, *E. faecalis* and *E. faecium* obtained in 2005 (prior to the introduction) with those recovered in 2007 (one year after the introduction).

**Methods:** A total of 1,506 isolates (610 *S. aureus*, of which 309 were MRSA, 310 *S. epidermidis*, 299 *E. faecalis*, 287 *E. faecium*) collected in 2005 (n=765) and 2007 (n=741) were included. Agents tested were tigecycline, doxycycline, oxacillin, amoxicillin-clavulanic acid, moxifloxacin, gentamicin, linezolid, vancomycin and others. MICs were determined by the broth microdilution method according to the standard of the guideline EN ISO 20776-1 in a central laboratory and interpreted by EUCAST criteria. Changes in resistance over time were assessed.

**Results:** Comparing data of 2005 and 2007, rates of resistance (%) were as follows: MSSA – tigecycline 0/0, doxycycline 3/3, moxifloxacin 14/12, gentamicin 7/8, linezolid 0/0, vancomycin 0/0; MRSA – tigecycline 0/0, doxycycline 6/4, moxifloxacin 91/95, gentamicin 23/13, linezolid 0/0, vancomycin 0/0; *S. epidermidis* – oxacillin 83/83, tigecycline 0/0, doxycycline 10/9, moxifloxacin 42/52, gentamicin 65/56, linezolid 0/0, vancomycin 0/0; *E. faecalis* – tigecycline 0/0, amoxicillin-clavulanic acid 0/1, high-level (HL) gentamicin 38/39, linezolid 0/0, vancomycin 0/0; *E. faecium* – tigecycline 0/0, amoxicillin-clavulanic acid 92/93, HL gentamicin 43/37, linezolid 1/0, vancomycin 0/18. MIC90 values of tigecycline remained unchanged with ≤0.125, ≤0.125, ≤0.125 mg/L for MSSA, MRSA, *S. epidermidis*, *E. faecalis* and *E. faecium*, respectively.

**Conclusion:** The in vitro activity of tigecycline against staphylococci and enterococci did not change compared to pre-marketing baseline values. In contrast, susceptibility to gentamicin increased among MRSA, while susceptibility to vancomycin decreased significantly among *E. faecium* isolates.

**P1076** Susceptibility of *Staphylococcus aureus* nosocomial isolates in Russia: five-year trends

A. Dekhmich*, A. Nikulin, N. Ivanchik, O. Kretshikova, R. Kozlov (Smolensk, RU)

**Objectives:** To evaluate antimicrobial susceptibility trends in nosocomial *S. aureus* isolates in different regions of Russia.

**Methods:** A total of 1456 clinical strains were collected during multicentre studies (24 cities, 29 centres) in two time periods: 2001–2002 and 2006–2007. Susceptibility testing to 13 antimicrobials was performed by CLSI agar dilution method. CLSI 2008 criteria were used for the interpretation of susceptibility testing results (with the exception of fusidic acid, for which the criterion of French Society for Microbiology was applied).

**Results:** Overall, 41.7% of strains were MRSA. Oxacillin resistance rates increased from 33.4% in 2001–2002 to 54.4% in 2006–2007. The MIC and resistance rates to other non-β-lactam antibiotics are presented in the Table.

**Conclusion:** Linezolid, vancomycin, mupirocin, trimethoprim/sulfa-methoxazole and fusidic acid retained high in vitro activity against...
nosocomial Staphylococcus aureus strains in Russia. Resistance to fluoroquinolones, lincosamides, macrolides, aminoglycosides, tetracyclines, fusidic acid, rifampicin and chloramphenicol substantially increased during five years period.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>2001–2002 (n=879)</th>
<th>2006–2007 (n=577)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC50 (mg/l)</td>
<td>MIC90 (mg/l)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8</td>
<td>128</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.125</td>
<td>256</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.5</td>
<td>256</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.5</td>
<td>256</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5</td>
<td>128</td>
</tr>
<tr>
<td>Co-trimoxazole*</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Notes: *MIC values are indicated for trimethoprim.

**Conclusion:** Overall resistance rates in S. aureus, including MRSA incidence, in the community settings are relatively low in Russia. It is necessary to perform further investigation to assess the dynamics and epidemiology of resistance.

**New antimicrobials against Gram-positives**

**P1077** Susceptibility of Staphylococcus aureus in community settings: first Russian surveillance

A. Nikulin, A. Dekhmich*, N. Ivanichik, R. Kozlov (Smolensk, RU)

**Objectives:** To evaluate antimicrobial susceptibility of community-onset strains of S. aureus in different regions of Russia.

**Methods:** A total of 417 clinical community-onset S. aureus strains were collected from 12 cities during multicentre study. Susceptibility testing to 15 antimicrobials was performed by CLSI agar dilution method. CLSI 2008 criteria were used for the interpretation of susceptibility testing results (with the exception of fusidic acid, for which the criterion of French Society for Microbiology was applied).

**Results:** Only 3.8% of strains were resistant to oxacillin. In general, all tested antibimisrobials revealed good activity against MSSA. At the same time MRSA strains were much less susceptible than MSSA to fluoroquinolones, macrolides, lincosamides, tetracyclines, aminoglycosides and chloramphenicol. Overall MICs and resistance rates of tested strains are presented in the Table.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC50 (mg/l)</th>
<th>MIC90 (mg/l)</th>
<th>S (%)</th>
<th>I (%)</th>
<th>R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>8</td>
<td>64</td>
<td>73.6</td>
<td>0</td>
<td>26.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.5</td>
<td>1</td>
<td>93.3</td>
<td>1.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.06</td>
<td>0.125</td>
<td>95.4</td>
<td>0</td>
<td>4.6</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.25</td>
<td>512</td>
<td>81.8</td>
<td>1.0</td>
<td>17.2</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>0.06</td>
<td>0.125</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.5</td>
<td>1</td>
<td>96.3</td>
<td>0</td>
<td>3.8</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.125</td>
<td>0.25</td>
<td>96.2</td>
<td>0</td>
<td>3.8</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>0.125</td>
<td>0.25</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>0.25</td>
<td>0.5</td>
<td>99.5</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0.5</td>
<td>0.5</td>
<td>96.2</td>
<td>0</td>
<td>3.8</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.015</td>
<td>0.015</td>
<td>98.8</td>
<td>0</td>
<td>1.2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5</td>
<td>32</td>
<td>84.4</td>
<td>0.2</td>
<td>15.3</td>
</tr>
<tr>
<td>Co-trimoxazole*</td>
<td>0.06</td>
<td>0.06</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes: *MIC values are indicated for trimethoprim.
exposure to antimicrobial agents was examined by scanning electron microscopy.

**Results:** Each of the porphyrins caused rapid and virtually complete loss of ATP and potassium from the cell within 5 minutes of exposing *S. aureus* to lethal concentrations of the agents. XF-70 and XF-73 had more pronounced initial effects on the membrane potential than DPD-S288 and DPD-S137. In contrast, DPD-207 promoted greater leakage of magnesium than XF-70 or XF-73. Pre-treatment of bacteria with the ionophore valinomycin (which dissipates the membrane potential) delayed the release of ATP by XF-70, but valinomycin had little effect on the kinetics of ATP release mediated by XF-73 and DPD-207. The gross morphological appearance of *S. aureus* exposed to the porphyrin agents was unaffected compared to drug-free controls.

**Conclusion:** The anti-staphylococcal activity of the metallo- and nonmetalloporphyrins studied here can be attributed to their effects on the integrity of the cytoplasmic membrane and leakage of potassium and ATP appear to be particularly good correlates of membrane damage and bactericidal activity. However the insertion of these agents into the staphylococcal membrane may differ based upon the apparent requirement of a membrane potential for the binding of XF-70 and differences in the ability of the porphyrins to dissipate the membrane potential and release magnesium.

**P1080** In vitro activity of delafloxacin against methicillin-resistant *Staphylococcus aureus* from the United States, Europe and Asia

E. Burak*, S. Hopkins, C. Pillar, L. Lawrence (New Haven, Chantilly, US)

**Objectives:** Delafloxacin (DFX) is an investigational fluoroquinolone with excellent activity against a variety of Gram-positive bacteria, including quinolone-resistant methicillin-resistant *Staphylococcus aureus* (MRSA). We evaluated the in vitro activity of DFX against MRSA from the United States (US), Europe, and Asia. The collection included prevalent clones of MRSA (e.g. USA100 and USA300) as well as isolates from regions, although isolates with higher DFX MIC values were more frequently encountered in Asia. Against characterised epidemic MRSA isolates, including 2 strains with reduced susceptibility to VAN, DFX maintained potent activity superior to that of levofloxacin.

**Methods:** Minimum inhibitory concentration (MIC) values were determined by broth microdilution according to CLSI methodology. Comparator agents included levofloxacin (LVX), linezolid (LNZ), daptomycin (DAP), vancomycin (VAN), oxacillin (OXA), clindamycin (CLI), erythromycin (ERY), and tigecycline (TIG).

**Results:** MIC values (mcg/mL) are presented below. MIC50/MIC90 values for DFX were 0.12/0.25 mcg/mL against strains from Europe (n=387), 0.12/0.25 mcg/mL against strains from Asia (n=97) and 0.12/0.5 mcg/mL against strains from the US (n=477). In comparison, LFX was at least 16-fold less active than DFX against these geographically grouped strains. Against 16 genetically characterised strains of MRSA, including USA100, USA300, and strains with reduced susceptibility to VAN, MIC50/MIC90 values for DFX and LVX were <0.004/0.25 mcg/mL and 0.5/16 mcg/mL, respectively.

**Conclusions:** DFX was more potent than LVX against MRSA, regardless of geographic region. The activity of DFX was largely consistent across regions, although isolates with higher DFX MIC values were more frequently encountered in Asia. Against characterised epidemic MRSA isolates, including 2 strains with reduced susceptibility to VAN, DFX maintained potent activity superior to that of levofloxacin.

**P1081** A novel antibacterial protein which shows rapid bactericidal activity against MRSA in the presence of other antibiotics


**Objectives:** SASP are small acid-soluble spore proteins which, when expressed in vegetative bacteria, are rapidly bactericidal. SASP bind to bacterial DNA in a non-sequence-specific manner and inhibit DNA replication and transcription. SASPject technology consists of using specifically tailored delivery vectors to deliver SASP genes to selected target bacteria. In practice, antibiotics are sometimes co-administered and Phico’s *S. aureus* specific SASP, PT1.2, could be suitable for such use, particularly where efficacy against MRSA is required. To assess whether SASP efficacy is compromised in the presence of other antibiotics, time-kill assays were conducted with PT1.2 in combination with 5 separate antibiotics at various concentrations.

**Methods:** Dilutions of an overnight culture of EMRSA 15 (CC22 SCCmeCIV) (10⁷ cfu/mL final concentration) were incubated with PT1.2 (1.5×10⁴ pfu/mL final concentration) in Luria-Bertani broth supplemented with calcium, together with each of vancomycin (Van), tetracycline (Tet), linezolid (Lin), ciprofloxacin (Cip) or rifampicin (Rif) at 0.1, 0.3, 1 or 3×MIC. Controls comprised each antibiotic alone and PT1.2 alone. The cultures were incubated at 37°C and samples were taken at 0, 0.25, 0.5, 1, 2, 6 and 24 hours to assess cell viability.

**Results:** SASP was rapidly bactericidal against MRSA, causing a 3–4-log drop in viability within 15 minutes. The rate and extent of kill was equal in all cultures containing PT1.2, showing that Van, Tet, Lin, Cip and Rif, between 0.1 and 3×MIC did not affect the efficacy of SASP against MRSA.

**Conclusions:** SASP is equally effective in the absence or presence of antibiotics with varying mechanisms of action, covering inhibition of cell wall biosynthesis (Van), protein synthesis (Tet, Lin), mRNA synthesis (Rif), and DNA replication (Cip). Thus PT1.2 could be used in combination with these (or potentially other) antibiotics, or when other antibiotics are present, without affecting efficacy.

**P1082** Studies on resistance development to LTX-109, a novel antimicrobial peptide

I. Morrissey*, A. Fugelli, W. Stensen, J.S. Svendsen (Fordham, UK; Tromso, NO)

**Objectives:** LTX-109 (LTX) is a novel broad-spectrum antimicrobial peptide currently being developed as a topical agent for the treatment of skin infections. In vitro studies have shown LTX activity is unaffected by resistance to established antibiotics. We assessed in vitro resistance development using LTX.

**Methods:** Resistance to LTX was compared with fusidic acid (FUS), a commonly used topical antibacterial agent using the following *S. aureus* strains: ATCC 29213 – susceptible reference strain; ATCC 433300 – methicillin-resistant; Mu50 – vancomycin-intermediate; VR531 – fully vancomycin-resistant and GP06 – teicoplanin-intermediate. Spontaneous resistance to LTX was assessed by subculture on agar plates in the presence of 2, 4, 8× and 16× MIC using 1 mL suspensions of bacteria at ~10¹⁰ per mL. Selection and amplification of resistance was determined by 14 serial passages in a macrobroth culture containing 0.5×MIC.

**Results:** Using selection on agar no resistant mutants were obtained with LTX. In contrast, each experiment using FUS resulted in confluent growth. Raised MICs were confirmed on each occasion by subculturing onto selective plates containing FUS at the original selecting concentration.

During serial passage, fusidic acid MIC increased sharply. In stark contrast, LTX showed little change in MIC over the 14 passages (P0 to P14, see Table).
New antimicrobials against Gram-positives

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (mg/L)</th>
<th>FUS</th>
<th>LTX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P0</td>
<td>P14</td>
<td></td>
</tr>
<tr>
<td>29213</td>
<td>0.06</td>
<td>512</td>
<td>2</td>
</tr>
<tr>
<td>433300</td>
<td>0.06</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>VRS1</td>
<td>0.06</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>Mu50</td>
<td>0.03</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>GP06</td>
<td>1</td>
<td>128</td>
<td>2</td>
</tr>
</tbody>
</table>

These data concur with the spontaneous resistance results indicating that resistance to LTX109 is not observed using either method of analysis.

**Conclusion:** LTX109 may be a valuable new agent for treating *S. aureus* infections, with a low propensity for resistance development in vitro. Further studies are warranted.

**P1083** Specific anti-staphylococcal activity of AFN-1252, a novel fatty acid synthesis inhibitor


**Objectives:** AFN-1252, a new molecular entity and lead clinical candidate, is a potent inhibitor of staphylococcal FabI (enoyl-ACP reductase [ENR]), an essential enzyme in bacterial fatty acid synthesis. AFN-1252 is currently in Phase 1 studies as an oral, specific-spectrum anti-staphylococcal antibiotic. Due to AFN-1252’s specificity for FabI, it is expected that bacterial species possessing alternate ENR forms will not be susceptible to its inhibition. To further assess the AFN-1252 spectrum, a phylogenetic analysis of bacterial ENR was performed and in vitro activities against staphylococci, and other aerobic and anaerobic bacteria were determined.

**Methods:** Phylogenetic trees were constructed with PHYLIP 3.52 using protein sequences from the NCBI Refseq database. Bacterial strains for susceptibility testing included 3,333 recent clinical isolates from 12 Canadian medical centres and characterised VISA and VRSA strains from NARSA. MICs were determined using CLSI methods.

**Results:** Four distinct bacterial ENR isozymes (FabI, FabL, FabV, FabK) were delineated by phylogenetic analyses. Of the 123 representative aerobic and anaerobic species analyzed, only 44 species had FabI only. For species that had FabI only, the FabI amino acid sequence identity was divergent compared to *Staphylococcus* spp. Other species had alternate or more than one form of ENR. AFN-1252 showed highly potent activity (MIC90 of 0.016 μg/ml) against all staphylococci including both susceptible and all drug resistant strains (e.g. MRSA, MRSE, VISA), but poor or no activity against all other aerobic and anaerobic Gram-negative and -positive species tested.

**Conclusions:** AFN-1252 showed a highly potent and selective spectrum of anti-staphylococcal activity. Species non-susceptible to AFN-1252 had either a non-FabI ENR enzyme, multiple ENR forms or an essential FabI that was highly divergent from the *Staphylococcus* spp. enzyme. The AFN-1252 specific-spectrum highlights the lack of resistance selection of AFN-1252 within the normal bacterial flora. The apparent lack of activity against common gut and skin flora also highlights its potential safety benefits including fewer adverse effects due to antimicrobial therapy such as diarrhoea, antibiotic induced colitis, *C. difficile* infections and candidiasis. These data support the continued development of AFN-1252 as a safe, targeted, oral therapy against staphylococcal infections.

**P1084** In vitro activity of AFN-1252 against MRSA in mouse and human serum

N. Kaplan*, R. Hinshaw, G. Zarenko, B. Hafkin (Toronto, CA; Kalamazoo, US)

**Objectives:** AFN-1252, a new molecular entity and lead clinical candidate, is a potent inhibitor of staphylococcal FabI (enoyl-ACP reductase), an essential enzyme in bacterial fatty acid synthesis. AFN-1252 is currently in Phase 1 studies as an oral, specific-spectrum anti-staphylococcal antibiotic. Previous AFN-1252 in vitro human plasma protein binding studies showed values ranging from 89% to 98% using ultrafiltration or equilibrium dialysis methods. To better define human PK/PD targets, including desired free AFN-1252 plasma concentrations, the effects of mouse and human serum on in vitro killing activity of AFN-1252 were determined.

**Methods:** *Staphylococcus aureus* 1659 (HA-MRSA) was used as test organism. Pooled mouse and human sera were both decomplemented by heat inactivation and filter-sterilised. Time-kill experiments using standard techniques were conducted in Mueller Hinton Broth diluted (v/v) with 0%, 25%, 50%, 75% or 90% serum. AFN-1252 was tested at concentrations ranging from 4 to 64 times the MIC. Plots of the change in log CFU/ml (compared to time 0) versus AFN-1252 concentration at various time points were fitted using inhibitory effect models. Apparent plasma protein binding values were calculated from the ratios of AFN-1252 concentrations required to achieve defined levels of bacterial killing in serum versus no-serum controls.

**Results:** The maximum serum concentration that supported adequate bacterial growth and killing effects was 75%. Under these conditions AFN-1252 showed apparent human plasma protein binding values of 92.3%, 93.8% and 94.4% at the 0 log kill (stasis), −1 log kill and −1.5 log kill (compared to time 0) points, respectively. The apparent mouse plasma protein binding values were very similar to the human values.

**Conclusions:** An apparent plasma protein binding value of approximately 95% was determined in both mouse and human serum. The time-kill experiments in mouse and human serum greatly assisted in defining a free drug concentration required to achieve bacterial killing in serum and provide a viable alternative to in vitro analytical methods. In addition these data suggest that AFN-1252 PK/PD parameters (expressed as total drug concentrations) derived from mouse infection models may be directly applicable to human infections.

**P1085** MUT056399: a novel antibacterial against methicillin-resistant staphylococci

S. Escaich*, F. Moreau, E. Malacain, S. Floquet, A. Walton, V. Sam-Sambo, V. Vongsouthi, L. Durant, C. Soulama, F. Faivre, Y. Bonna, M. Oxoby, V. Geruss (Romainville, FR)

**Objectives:** MUT056399 is a novel antimicrobial agent targeting the essential enzyme FabI which catalyses the final step of bacterial fatty acid elongation cycle. In this study, the biochemical activity of MUT056399 is presented along with its in vitro antibacterial activity.

**Methods:** IC50s were measured on purified FabI of *Staphylococcus aureus*. MIC testing and time-kill studies were performed according to CLSI methodologies. Cytotoxicity was measured using the HEPG2 cell viability assay.

**Results:** MUT056399 is a highly potent slow binding inhibitor of FabI of *S. aureus* with an IC50 of 13μM. MUT056399 displayed an antibacterial spectrum consistent with specific FabI inhibition: a high potency against methicillin susceptible and resistant staphylococci (mode MIC ≤ 0.06 μg/ml), an intermediate activity against *Escherichia coli*, and no activity against FabK containing bacteria (streptococci and enterococci). The MIC of MUT056399 on agar medium against 10 *S. aureus* strains was not significantly influenced by pH, divalent cations, NaCl and incubation temperature. It was only slightly influenced by inoculum size (2-fold when raised from 105 to 106 CFU/spot). Time kill studies showed a time-dependent mechanism of killing and a slow bactericidal effect for *S. aureus* ATCC 29213. Frequency of resistance was 2.5 × 10−9 at 4×MIC. MUT056399 did not show any cytotoxicity for eukaryotic cells up to 32 μg/ml.

**Conclusion:** MUT056399 is a highly potent anti-staphylococcal agent with a new mechanism of action targeting FabI. It was particularly active against methicillin susceptible and resistant *S. aureus* strains with stable MICs among testing conditions, a slow bactericidal activity, low frequency of resistance at 4×MIC, and no cytotoxicity up to 32 μg/ml.
Farnesol as a prospective antimicrobial agent against Staphylococcus epidermidis

F. Gomes*, N. Cerca, P. Teixeira, R. Oliveira (Braga, PT)

Objectives: Staphylococcus epidermidis is now among the most important pathogenic agents responsible for bloodstream nosocomial infections and for biofilm formation on indwelling medical devices. Its increasing resistance to common antibiotics is a challenge for the development of new antimicrobial agents. Accordingly, the goal of this study was to evaluate the effect of farnesol, a natural sesquiterpenoid, on Staphylococcus epidermidis biofilm cells and compare this one with the effect of vancomycin, one of the most frequently used antibiotics to treat resistant nosocomial infections. Another aim of this work was to determine if subjecting S. epidermidis cells to farnesol they acquire resistance.

Methods: A 24 h kinetic study was performed using vancomycin at the peak serum concentration (40 mg/L) and farnesol at concentrations of 30, 100, 200 and 300 microM. The growth inhibition effect of farnesol and vancomycin on biofilm cells of S. epidermidis was assessed by XTT (the reduction of this tetrazolium salt is a measure of cellular activity and is easily assessed by colorimetry) and Crystal Violet, which measures total biomass of biofilm. The biofilm cells were analysed by confocal laser scanning microscopy after being stained with Live/Dead. Resistance to farnesol and vancomycin was tested growing S. epidermidis planktonic cells in sub-inhibitory concentrations of farnesol and vancomycin and then subjecting these cells to inhibitory concentrations of both antimicrobial agents during 24 hours. After that, cellular activity was assessed by XTT. This was repeated for 5 consecutive days.

Results: Both tested agents act at the cell wall level, vancomycin inhibits the biosynthesis of bacterial cell wall, while farnesol is considered to disrupt the normal barrier function of the cell membrane. Interestingly, farnesol at a concentration higher than 200 microM displayed the same or higher effectiveness of vancomycin at peak serum concentration. In fact, the response of the strains tested was very similar for both farnesol (>200 microM) and vancomycin. Regarding cells resistance to farnesol, the results point out to a slight increase of tolerance but not to an acquired resistance, because the percentage of inhibition was steady along the time.

Conclusions: Overall, the results indicate a potential antibacterial effect of farnesol against S. epidermidis, and therefore the possible action of this molecule on the prevention of S. epidermidis related infections.

The potential of the breakdown products of casein by Lactococcus lactis strain 146 as an inhibitory therapeutic agent(s) for MRSA

M. Al-Mahrous*, M. Alqamber, J. Burnie, M. Upton, J. Tagg (Manchester, UK; Dunedin, NZ)

Objective: lactococci possess a proteolytic system that can release free amino acids, peptides and oligopeptides from casein (milk protein). The proteolysis involves the action of cell wall-associated peptidases (CWAP) and subsequent hydrolysis is carried out by several enzymes found in the cell envelope, which can eventually be taken up by the bacteria. This fermentation process results in milk diary’s flavour and proteinacious end-products. The objective of the current study was to investigate the end-products of casein degradation by Lactococcus lactis strain 146, as inhibitory agents for MRSA.

Methods: 1. Investigation of the end-products of L. lactis strain 146 was performed using plate-diffusion method from casein-containing minimum essential media (MEM).
2. Casein-free MEM was used as a negative control for the inhibitory end-products, on which alternative growth factors were included.

3. Purification and/or concentration of the end-products in broth supernatants was carried out using ammonium-sulphate precipitation, XAD-2 resin separation, cation-exchange; then C18 reverse-phase chromatography.
4. MALDI TOF/TOF mass-spectrometry (MS) was used for mass analysis.
5. A range of published and/or designed primers was used for PCR of gene(s) responsible for the synthesis of CWAP in strain 146.
6. The gene was cloned using T-tailed vector; and then sequenced.

Results: Supernatant from casein-containing media displayed activity against MRSA, but not the casein-free media. The reversed-phase HPLC profile of the processed active fractions revealed several peptide species (Fig. 1). In addition, determining the mass of the peptides with MS showed that they were seized in a window of 0.9 and 5 kD. Among the tested primers, BG95/146CEP–invlwoer1; BG97/146CEP–invlwoer1; BG95/4CA showed positivity with strain 146 on PCR. However, cloning was successful; the sequence data of the vector still needs further analysis.

Conclusion: The effect of the breakdown-products of casein by L. lactis strain 146 against MRSA suggests the potency of these peptides as future therapeutic agents for treating highly drug-resistant Staphylococcus aureus, on which the cloned vector or the sue of strain 146 can be a powerful biological tool for the breakdown of casein by its CWAP. Up to our knowledge, this is the first study that discusses casein breakdown products by L. lactis and their activity against staphylococci.
8. Biological activities were tested using spot-on-loan assay.

Results: The biological activity of A345 is heat-stable and displaying specificity for the closely-related S. aureus. The high ammonium-sulphate saturation (more than 80%) needed for precipitating A345 suggests its small mass. The cationic-exchange chromatography (PH 5.2) and the late elution from C18 column suggest the cationic nature of A345. MALDI TOF/TOF showed 4 species and sized the mass in a window between 1500.2 and 3200.7 Da. This suggests further purification using high resolution HPLC to eliminate, if any, unrelated species. The Electron microscopy diagnosis reveals a clear damage in the cell wall (Figure 1).

Figure 1. Thin section of EMRSA-15 strain A208 cell wall after incubation with the inhibitory substance A345 for 24 h at 37ºC. An example of a ruptured wall is seen.

Conclusion: 1. The biological activity of the highly-purified extract of the heat-stable small mass inhibitory agent A345, which shows specific inhibitory activity against Epidemic MRSA-15 and strains of MSSA, suggests its nature as a bacteriocin, possibly of Class-I. 3. The cation-exchange separation and late elution of A345 from C18 reverse-phase column suggest that it is a hydrophobic in nature. 4. Based on the electron microscopy diagnosis, A345 shows obvious damage to the protective cell wall of the sensitive indicators. This postulates the binding of the hydrophobic A345 to negatively-charged lipid-II in the cell-membrane resulting in its lysis. 6. A345 could have potential as topical therapeutic agents for treating highly drug-resistant staphylococcal infections.

Results: 135 treatment-emergent AEs were reported by 26 subjects as indicated below. The most common AEs reported following administration of TR-701 were nausea and headache (5 subjects each [20.8%]), and stomach discomfort (4 subjects [16.7%]). No clinically significant changes or findings were noted in vital sign measurements, physical examinations, or 12-lead ECGs. However, there were clinically significant values and changes in some laboratory evaluations. 4 subjects were discontinued due to laboratory AEs: 2 subjects (1 receiving 400 mg TR-701 and 1 receiving linezolid) had decreased reticulocytes, 1 subject receiving 400 mg TR-701 had a low WBC count present at baseline which further decreased, and 1 subject receiving 200 mg TR-701 had elevated liver ALT values (5 times ULN). There were no deaths or SAEs reported.

Conclusions: Multiple doses of 200 mg or 300 mg TR-701 through 21 days were well-tolerated. Multiple doses of 400 mg TR-701 presented a slightly higher incidence of AEs. TR-701 400 mg QD and linezolid 600 mg BID presented comparable effects on haematologic parameters.

New antimicrobials against Gram-positives

P. Bien, K.A. Munoz, P. Prokocimer*, J. Bohn (San Diego, Madison, US)

Objectives: TR-701, a novel oxazolidinone prodrug antibiotic that is rapidly converted in vivo by blood and tissue phosphatases, to the microbiologically-active molecule TR-700. TR-700 is active against Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE). A randomised, double-blind, placebo-controlled, single ascending oral dose study was performed to assess the safety, tolerability, and PK of TR-701 in healthy adult subjects.

Methods: 40 healthy male and female subjects were enrolled in this double-blind, sequential-dose escalation study. Cohorts of 8 subjects (6 active and 2 placebo) received a single dose of 200, 400, 600, 800, or 1200 mg oral TR-701 after a 10-hr fast. Safety was assessed via adverse events, physical examination, ECG and laboratory evaluations. Adverse events (AEs) were solicited proactively by asking multiple “how do you feel?” questions.

Summary of subjects with adverse events after single ascending oral doses of TR-701

<table>
<thead>
<tr>
<th>Placebo</th>
<th>TR-701 200mg</th>
<th>TR-701 300mg</th>
<th>TR-701 400mg</th>
<th>Placebo</th>
<th>BID 600mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=8</td>
<td>n=30</td>
</tr>
<tr>
<td>Any AE</td>
<td>5 (62.5) 5 (62.5) 5 (62.5) 1 (12.5)</td>
<td>5 (62.5) 5 (62.5) 5 (62.5) 1 (12.5)</td>
<td>5 (62.5) 5 (62.5) 5 (62.5) 1 (12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>1 (12.5)</td>
<td>2 (25)</td>
<td>3 (37.5)</td>
<td>1 (12.5)</td>
<td>2 (25) 1 (12.5)</td>
</tr>
<tr>
<td>Moderate</td>
<td>5 (62.5) 5 (62.5) 5 (62.5) 1 (12.5)</td>
<td>5 (62.5) 5 (62.5) 5 (62.5) 1 (12.5)</td>
<td>5 (62.5) 5 (62.5) 5 (62.5) 1 (12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment-related AEs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>during first 7 days of drug administration</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AE leading to study discontinuation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serious AEs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Effects of food on the pharmacokinetics of TR-701, a novel oxazolidinone prodrug antibiotic, in healthy adult subjects

K. A. Munoz, P. Bien, P. Prokokheimer*, M. Berry, C. Bethune (San Diego, Madison, US)

Objectives: TR-701 is a novel oxazolidinone prodrug antibiotic that is rapidly converted in vivo to the microbiologically-active molecule TR-700. TR-700 is active against Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE). A randomised, open label, 2-sequence, 2-period, 2-treatment crossover, single oral dose study was performed to evaluate the safety, tolerability, and effect of food on the pharmacokinetics (PK) of TR-701 in 12 healthy adult subjects.

Methods: Subjects received a single oral dose of 600 mg TR-701 after a 10-h fast or after eating a high-fat breakfast preceded by a 10-h fast.

Results: TR-701 was rapidly and extensively converted to TR-700. In the fed state, the mean TR-700 Cmax was significantly (26%) lower than that observed in the fasted state. A 6-hour delay in TR-700 median Tmax was observed in the fed compared to the fasted state. The mean TR-700 AUC(0-24) and AUC(0-∞) values in the fed state were similar (within 2%) to the corresponding values in the fasted state. TR-701 600 mg was well-tolerated and no significant clinical or laboratory abnormalities were reported. 8 mild AEs were reported by 6 subjects, with 5 reported in the fed state and 3 reported in the fasted state. Two treatment-related AEs, gastro-oesophageal reflux disease (fed) and insomnia (fasted), were reported by a single subject each. There were no apparent trends in AEs and no AE was reported more than once. No clinically significant changes or findings were noted from clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead ECGs. Overall, changes in safety evaluations were unremarkable.

Conclusions: TR-701 is safe and well tolerated in healthy volunteers at single doses up to 1200 mg.

Ceftaroline activity tested against common organisms

Objectives: Ceftaroline is the bioactive metabolite of ceftaroline fosamil, a N-phosphonoamino water-soluble cephalosporin prodrug. Ceftaroline is active against methicillin-resistant S. aureus (unaffected by the strain to linezolid), probably in relation with its extensive accumulation within cells and its higher intrinsic activity (lower MIC values).

Methods: Unique (1 per patient) clinically significant isolates of S. aureus (2168), β-haemolytic streptococci (BHS; 172), viridans group streptococci (VGS; 86), and E. faecalis (409) were consecutively collected from 24 medical centres in 10 European (EU) countries, Turkey and Israel in 2008. The strains were tested for susceptibility (S) by the CLSI broth microdilution method (M7-A7; M100-S18) against ceftaroline and numerous antimicrobials currently available for SSSI treatment.

Results: 25.4% of S. aureus isolates were MRSA. Ceftaroline was very active against methicillin-susceptible S. aureus (MSSA; MIC90, 0.25 mg/L) and MRSA (MIC90, 2 mg/L). Against MSSA, ceftaroline was 16-, eight- and four-fold more potent than ceptriaxone (CRO), linezolid (LZD) and vancomycin (VAN), respectively. The highest ceftaroline MIC among MSSA was 1 mg/L, and 90.9 and 99.9% of strains were inhibited at ≤0.25 and ≤0.5 mg/L, respectively. Among MRSA, 97.8% of strains were inhibited at 2 mg/L of ceftaroline. All MRSA strains with ceftaroline MICs of ≥2 mg/L (12 strains at 4 mg/L) were found in Greece (2 centres). MRSA showed high rates of resistance (R) to levofloxacin (LEV; 84.4%) and clindamycin (CLI; 35.1%). Only 85.8% of MSSA strains were quinupristin/dalfopristin-S. Against BHS, ceftaroline was
64- and 32-fold more potent than LZD and VAN, respectively, and all strains were inhibited at ≤0.06 mg/L of ceftaroline. VGs were very S to ceftaroline, while 79.1 and 90.7% of strains were S to penicillin and CRO, respectively. More than 90% of E. faecalis, including all VAN-R isolates (VRE) were inhibited by ≤8 mg/L of ceftaroline. Four of 5 VRE were from Greece.

**Conclusions:** Ceftaroline demonstrated broad-spectrum and high activity against the most common SSSI Gram-positive pathogens, including MRSA, isolated in EU medical centres in 2008. This favourable antimicrobial profile demonstrates that ceftaroline is a promising anti-MRSA therapeutic option in the treatment of SSSI.

**Organism (no. tested) BPR MIC (mg/L) % Susceptible**

**CRO** 0.25/NA 4/100 0.25/100 0.5/100 1.0/100 2/100
**LEV** 0.25/NA 4/100 0.25/100 0.5/100 1.0/100 2/100
**CLI** 0.25/NA 4/100 0.25/100 0.5/100 1.0/100 2/100
**VAN** 0.25/NA 4/100 0.25/100 0.5/100 1.0/100 2/100

**Conclusions:** Bacteriologic pathogens recently collected (2008) from CARTI in EU medical centres were very S to ceftaroline, including community-acquired MRSA, PEN-R SPN, and other R strains. This favourable antimicrobial profile places ceftaroline as a promising and potentially effective therapeutic option in the treatment of CARTI in the EU.

**Antimicrobial activity of ceftaroline against bacteria isolated in 2008 from community-acquired respiratory tract infections in European hospitals, including methicillin-resistant Staphylococcus aureus**

**H. Sader**, P. Rhomberg, R. Jones (North Liberty, US)

**Objectives:** To evaluate the potency and spectrum of ceftaroline tested against community-acquired respiratory tract infection (CARTI) pathogens. Ceftaroline, currently in phase III clinical development, is a novel N-phosphono prodrug cephalosporin that has high affinity to the bacterial transpeptidase, making it an attractive candidate for broad-spectrum therapy. Results show EC was nearly identical for the three agents (Table; 97–98% inhibited at ≤8 mg/L, respectively. Overall SA strains had MIC90 at 2 mg/L, however the MIC90 for oxacillin (OXA)-S strains was 4-fold lower (0.5 mg/L). Coverage against Gram-negative bacilli causing HA-RTI showed EC was nearly identical for the three agents (Table: 97–98% inhibited at ≤4 mg/L). Whereas FEP provided enhanced coverage against KSP (90% at ≤8 mg/L vs. 83% for BPR and 88% for CAZ), BPR and FEP were superior to CAZ against ESP. FEP was equal in potency to FEP (MIC90, 8 mg/L) and two-fold more potent than CAZ, although the % inhibited for these agents at ≤/2/≤/4/≤/8 mg/L was similar (67–92/60–90/66–87%, respectively).

**Conclusions:** BPR is a new β-lactam with antimicrobial activity against pathogens causing CA- and HA-pneumonia, similar to that of extended-spectrum cephalins but including MRSA. These characteristics warrant continued evaluation of BPR as empiric therapy for treating bacterial pneumonia.

**Antimicrobial activity of ceftaroline, a novel anti-methicillin-resistant S. aureus cephalosporin, tested against skin and skin-structure infection pathogens (North America)**

**R. Jones**, H. Sader, M. Janecek, P. Rhomberg (North Liberty, US)

**Objectives:** To establish ceftobiprole (BPR) an investigational parenteral cephalosporin in regulatory review for community- (CA) and hospital-acquired (HA) respiratory tract pathogens) potency and spectrum. BPR is active against MRSA and other Gram-positive and -negative pathogens, making it an attractive candidate for broad-spectrum therapy. Results assessing potency of BPR against commonly occurring CA- and HA-pneumonia pathogens in North America (NA) are presented.

**Methods:** A total of 5,108 non-duplicate isolates causing clinically-significant CA- and HA-pneumonia infections were collected from over 25 medical centres in NA participating in a BPR surveillance program (2005–2007). Susceptibility (S) testing was performed using CLSI methods (M7-A7, 2006) by the central monitoring laboratory. Results: BPR inhibited the CA-RTI pathogens HI and SPN at ≤0.25 and ≤1 mg/L, respectively. Overall SA strains had MIC90 at 2 mg/L, however the MIC90 for oxacillin (OXA)-S strains was 4-fold lower (0.5 mg/L). Coverage against Gram-negative bacilli causing HA-RTI showed EC was nearly identical for the three agents (Table: 97–98% inhibited at ≤4 mg/L). Whereas FEP provided enhanced coverage against KSP (90% at ≤8 mg/L vs. 83% for BPR and 88% for CAZ), BPR and FEP were superior to CAZ against ESP. BPR was equal in potency to FEP (MIC90, 8 mg/L) and two-fold more potent than CAZ, although the % inhibited for these agents at ≤/2/≤/4/≤/8 mg/L was similar (67–92/60–90/66–87%, respectively).

**Conclusions:** BPR is a new β-lactam with antimicrobial activity against pathogens causing CA- and HA-pneumonia, similar to that of extended-spectrum cephalins but including MRSA. These characteristics warrant continued evaluation of BPR as empiric therapy for treating bacterial pneumonia.
surveillance program (2005–2007). Identiﬁcations were conﬁrmed by the central monitoring laboratory and all isolates were susceptibility (S) tested using CLSI methods.

Results: BPR inhibited all SA, EF and BHS at ≤2, ≤1 and <0.12 mg/L, respectively. MIC90 values for oxacillin (OXA)-R SA strains were two-fold higher than for OXA-S strains (1 versus 0.5 mg/L). Coverage against EC was nearly identical for the three agents (Table; 97–98% inhibited at ≤4 mg/L). Whereas FEP provided enhanced coverage against KSP (90% at ≤8 mg/L vs. 83% for BPR and 88% for CAZ), BPR and FEP were superior to CAZ against ESP. Against PSA, BPR was equal in potency to FEP (MIC90, 8 mg/L) and two-fold more potent than CAZ, although the % inhibition for these drugs at ≤2/≤4/≤8 mg/L was similar (67–92/60–90/66–87%, respectively).

Conclusions: BPR is a new β-lactam with recognized activity against NA SSSI pathogens, similar to that of extended-spectrum cephalosporins but including the sporadic and MSSA isolates included in the study. In vitro activity of BPR as empiric therapy for SSSI, including Gram-negative pathogens.

<table>
<thead>
<tr>
<th>Species (no. tested)</th>
<th>MIC90 in mg/L (%) at ≤2/≤4/≤8 mg/L</th>
<th>BPR</th>
<th>CRF or CAZ</th>
<th>FEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (SA; 996)</td>
<td>1 (100/–/–)</td>
<td>≤2 (15/45/51)b</td>
<td>&gt;16 (40/57/76)</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa (PA; 100)</td>
<td>1 (100/–/–)</td>
<td>≤0.12 (100/–/–)</td>
<td>&gt;12 (96/100/–)</td>
<td></td>
</tr>
<tr>
<td>E. coli (EC; 99)</td>
<td>≤0.06 (98/97/97)</td>
<td>≤0.06 (100/–/–)</td>
<td>&gt;0.12 (100/–/–)</td>
<td></td>
</tr>
<tr>
<td>E. faecalis (EF; 69)</td>
<td>1 (100/–/–)</td>
<td>≤0.06 (100/–/–)</td>
<td>&gt;0.25 (100/–/–)</td>
<td></td>
</tr>
<tr>
<td>Bacteroides fragilis (BHF; 52)</td>
<td>≤0.12 (100/–/–)</td>
<td>≤0.12 (100/–/–)</td>
<td>&gt;0.12 (100/–/–)</td>
<td></td>
</tr>
<tr>
<td>Enterococcus spp. (ESR; 54)</td>
<td>8 (50/50/50)</td>
<td>16 (65/67/69)</td>
<td>4 (80/86/88)</td>
<td></td>
</tr>
<tr>
<td>Klebsiella spp. (KSP; 42)</td>
<td>≥0.5 (50/50/50)</td>
<td>0.12 (100/–/–)</td>
<td>&gt;0.25 (100/–/–)</td>
<td></td>
</tr>
<tr>
<td>P. mirabilis (13)</td>
<td>≥0.06 (100/–/–)</td>
<td>≤0.06 (100/–/–)</td>
<td>&gt;0.12 (100/–/–)</td>
<td></td>
</tr>
</tbody>
</table>

BPR = cefditoren; CAZ = cefazolin; FEP = cefepime.

**P1097** In vitro activity of cefditoren against a group of well-characterised staphylococci

S. Borbone*, F. Campanile, D. Bongiorno, S. Jeddari, C. Scuderi, S. Stephani (Catania, IT)

Objectives: In an effort to test the in vitro activity proﬁle and the antibacterial spectrum of cefditoren, a new cephalosporin particularly active against MRSA due to its high afﬁnity to PBP2a, we examined the susceptibilities of a group of well-characterised HA-MRSA, CA-MRSA, MSSA and CoNS isolates, responsible for documented cases of staphylococcal disease, in Italy.

Methods: MICs and MBCs were determined for cefditoren (BPR) and the main anti Gram-positive antimicrobials (vancomycin, teicoplanin, daptomycin, linezolid, synergic, and tigecycline) against 117 isolates of Staphylococcus spp.: 50 MRSA strains representative of the major epidemic clones (4 Archaic, 8 Iberian, 12 Italian, 4 Brazilian, 6 Rome, 7 sporadic, and 16 GS-MRSA strains); 8 CA-MRSA; 26 MSSA 20 MRCoNS, and 6 control strains.

Results: Cefditoren had a MIC90 value of 4 mg/L against the MDR Italian, Rome and Iberian clones; 2 mg/L against the Brazilian clone and the CoNS strains, while it was active at concentrations below 2 mg/L against the Archaic and GS-MRSA clones, the CA-MRSA strains and all MRSA. This conﬁrmed the study. In vitro activity of cefditoren is comparable to those of vancomycin (MIC90 2 mg/L), teicoplanin (MIC90 8 mg/L) and linezolid (MIC90 2 mg/L); good activity was demonstrated by daptomycin (1 mg/L), synergic (1 mg/L), and tigecycline (0.5 mg/L). Almost all strains showed MBC values similar or one-fold higher than their MIC values, except for few strains belonging to the Iberian, Rome, Brazilian and Italian clones, which paradoxically survived at higher concentrations of BPR.

Conclusion: Cefditoren is a very active bactericidal compound against multi-drug resistant hospital associated epidemic clones, and is also very active against emerging community-acquired strains possessing a complex virulence make-up. Clinical studies will conﬁrm its usefulness in the treatment of infections sustained by multi-resistant microorganisms.

**P1098** Efficacy and pharmacodynamic evaluation of CEM-101, a novel macrolide, in murine infection models

T.M. Murphy*, M. Gaffney, S. Little, R. Wu, A.M. Sloc, C. Ong, P. Fernandes (Waltham, Winter Park, Chapel Hill, US)

Objectives: To evaluate the in vivo efficacy of CEM-101 against Gram positive pathogens including community associated MRSA.

Methods: Efficacy was evaluated in both a subcutaneous abscess model as well as neutropenic thigh infection model. Abscesses were induced in CD-1 female mice by s.c. injection of *Streptococcus pneumoniae* or *S. pyogenes* mixed with cytodex beads. CEM-101 or comparator test articles were administered as a single oral dose two hours post infection with bioburden levels assessed at 48 hours post infection. In addition, the neutropenic thigh infection model was utilised to determine target organ effector activity after a single oral dose. CD-1 female mice were rendered neutropenic with cyclophosphamide pre-treatment. Mice were infected with *S. pneumoniae* (SPN) or *S. aureus* via IM injection into the right thigh. At 1.5 hours post infection, mice received treatment via oral gavage with CEM-101 ranging from 1 to 25 mg/kg. CFUs/gram of thigh were determined at initiation of treatment and at 24 hour post start of treatment. Subsequently, for a preliminary evaluation of PK-PD relationship, mice, infected with SPN, were treated with 4 doses of CEM-101 fractionated into 1, 2, 3, or 4 doses over a 24 hour period.

Single dose plasma PK was also performed.

Results: In the abscess, a 10 mg/Kg QD dose of CEM-101 demonstrated a 2.3 log10 decrease while clarithromycin only achieved a 0.9 log10 reduction from untreated mice against SPN. Similarly, a 2.9 log10 decrease was observed for CEM-101 against *S. pyogenes*; while clarithromycin demonstrated only a 0.5 log10 reduction. In the thigh model, CEM-101 demonstrated efficacy after a single oral dose against both susceptible and MRSA isolates. Evaluation of PK-PD demonstrated concentration dependent killing with increased bacterial reduction for the single oral dose over the fractionated cohorts. The effect of CEM-101 on bacterial burden was combined with free drug concentrations to predict the most likely PK-PD parameter. Cmax/MIC was the best predictor of in vivo efficacy with an r²=0.83.

Conclusions: CEM-101 demonstrated significant in vivo activity in a subcutaneous abscess and neutropenic thigh infection model. Preliminary PK-PD suggests concentration dependent killing with Cmax/MIC being the best predictor of efficacy against this isolate.

**P1099** Comparative activities of the novel ketolide CEM-101 and telithromycin towards towards *Streptococcus pneumoniae* resistant to macrolides from patients with confirmed community-acquired pneumonia

A. Lismong*, F. Van Bambeka, P. Tulkens (Brussels, BE)

Background and Aims: CEM-101) is a new macrolide-ketolide in development with activity against macrolides (ML)-resistant isolates. After 400 mg qD, it yields an AUC24h similar to that of telithromycin (3500 mgqD) and shows similar protein binding properties in human serum (about 15% free drug). Belgium is a country with high resistance of *Streptococcus pneumoniae* (SP) to ML (>35% for clarithromycin). Our aim was to compare the activity of CEM-101 to that of telithromycin (TEL) against ML-resistant strains of SP obtained from patients with confirmed CAP.

Methods: 29 first ML-R isolates (based on clarithromycin MICs determination; 19 MLSB, 10 M-phenotype based on erythromycin and clindamycin resistance dissociation) were selected (for which 6 were TEL-1 and 7 TEL-R based on EUCAST breakpoints [S ≤ 0.25 – R > 0.5]). MICs were determined by geometric microlodulation in CAMH broth + 2.5% lysed horse blood according to CLSI, using SP ATCC-49619 as a control.

Results: ATCC-49619 MICs were <0.008 mg/L for TEL and CEM-101. Data for ML-resistant isolates are shown in the Table.

Conclusions: In this Belgian collection of *S. pneumoniae* resistant to macrolides and isolated from confirmed CAP, CEM-101 shows globally
lower MICs compared to TEL, especially with respect to TEL-I and TEL-R isolates. CEM-101 has therefore the potential to stand as an alternative to telithromycin in areas with high ML resistance and emerging resistance to TEL.

### Methods:
A collection of 2006–2007 clinical isolates were S tested against 452 staphylococci and selected streptococci are described here.

### Results:
The potency of CEM-101 against each BHS serogroup was confirmed. The spontaneous frequency of resistance was determined by plating bacteria followed by dilution and incubation in antibiotic-free pre-warmed medium with bacterial enumeration on agar medium (37ºC, 48 hours). The PAE of NXL103 for S. pyogenes was determined by incubating bacteria for 2 hours with antibiotic followed by dilution and incubation in antibiotic-free pre-warmed medium with bacterial enumeration on agar medium (37ºC, 48 hours). PAE was defined as the difference in time required for antibiotic-treated bacteria to increase by 1-log10 versus bacteria not exposed to antibiotic. Spontaneous frequency of resistance was determined by plating bacteria on brain-heart agar containing 2, 4, or 8×MIC of antibiotic (37°C, 48–72 hours). MICs of mutant and parent strains were subsequently confirmed.

### Conclusions:
CEM-101 warrants further development for RTI and SSSI indications. CEM-101 was tested against a collection of 43 TEL-R (β-haemolytic streptococci (BHS)).

### Methods:
A total of 53 (1.3%) BHS were identified among 3,958 in the SENTRY Antimicrobial Surveillance Program (2003–2006) that were TEL-R (MIC, ≥2 mg/L). 43 strains (36 group A, 1 group C, 6 group G) were available for testing, from 20 hospitals in Europe (31 strains), North America (11) and Latin America (1). Susceptibility (S) testing used CLSI broth microdilution methods and 3 strains were erythromycin (ERY-R, clindamycin (CC)-S) requiring D-test. Nine comparison agents were tested (4 in Table).

Table. MIC distributions for CEM-101 as MLSβ-ketolide comparisons agents

### Antimicrobial Occurrences at MIC (mg/L):

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Occurrences at MIC (mg/L):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 &gt;4</td>
</tr>
<tr>
<td>CEM-101</td>
<td>4 0 1 18 10 6 4 0 0 0</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>0 0 0 0 0 0 8 16 19</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0 0 0 0 0 0 0 43</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>– – – 2 1 0 0 40</td>
</tr>
<tr>
<td>Q/D^*</td>
<td>– – – 37 6 0 0 0</td>
</tr>
</tbody>
</table>

^*Q/D = quprinistin/dalfopristin.

### Results:
The potency of CEM-101 against each BHS serogroup was the same with an overall MIC50 and MIC90 of 0.12 and 0.5 mg/L, respectively. CEM-101 activity was 32-fold (MIC50 comparisons) greater than TEL. All strains were ERY-R, but quinupristin/dalfopristin (Q/D) was 100% S. Three CC-S strains (S. pyogenes) were D-test (+) and 2 had (+) induction of CEM-101. The S rates for other comparators were: penicillin, tetracycline, ceftriaxone, amoxicillin/clavulanate, and levofloxacin (100.0%); and tetracycline (46.8%).

### Conclusions:
CEM-101 remained active against all TEL-R (MIC, ≥2 mg/L) BHS with all MICs ≤1 mg/L (MIC50, 0.12 mg/L). Highest occurrence of TEL-R strains was in Europe (greatest in Italy). CEM-101 warrants further development for infections caused by BHS.

### Antibacterial activity of NXL103 (linopristine-flopristine), in vitro post-antibiotic effect, and spontaneous frequency of resistance

**J. Pace**, P. Leauasse, A.M. Giraud, L. Luallade, I. Morrisse (Romainville, FR; London, UK)

### Objectives:
NXL103 (linopristine-flopristine) is an oral streptogramin which was recently evaluated in a Phase II clinical trial. Susceptibility of both community- (CA-MRSA) and hospital-associated (HA-MRSA) methicillin-resistant *Staphylococcus aureus* was evaluated against 160 clinical isolates and in vitro post-antibiotic effect (PAE) and spontaneous frequency of resistance have also been evaluated with selected strains.

### Methods:
Minimal inhibitory concentrations (MIC) were determined by CLSI broth microdilution method. In vitro post-antibiotic effect (PAE) was determined by incubating bacteria for 2 hours with antibiotic followed by dilution and incubation in antibiotic-free pre-warmed medium with bacterial enumeration on agar medium (37°C, 48 hours). PAE was defined as the difference in time required for antibiotic-treated bacteria to increase by 1-log10 versus bacteria not exposed to antibiotic. Spontaneous frequency of resistance was determined by plating bacteria on brain-heart agar containing 2, 4, or 8×MIC of antibiotic (37°C, 48–72 hours). MICs of mutant and parent strains were subsequently confirmed.

### Results:
NXL103 MICs ranged from 0.06–0.5 mg/L. NXL103 MIC90 was 0.25 mg/L for CA-MRSA and 0.5 mg/L for HA-MRSA. NXL103 was active against erythromycin A-resistant bacteria with MICs 2–4 fold lower than for linezolid, daptomycin, vancomycin, and quinupristin/dalfopristin. The PAE of NXL103 for S. aureus AS5155 and DEL4811 were 2 and 2.1 hours, respectively. First-step mutants of S. aureus ATCC 25923 were isolated at frequencies of 4.1×10⁻⁶ to 1.8×10⁻¹⁰ at
concentrations of 2 and 4×MIC (MIC increased from 0.25 to 2–4 μg/mL). No mutants were isolated at 8×MIC (2 mg/L) (frequency <2.9×10^-10). MICs of the mutants were similarly increased to pristinamycin and erythromycin A, but not to pefloxacin, chloramphenicol or tetracycline. No mutants of S. aureus AS5155 were isolated at concentrations of 2, 4, or 8×MIC (1, 2, and 4 μg/mL, respectively) (frequency <3.1×10^-10).

Conclusions: NXL103 exerts antibacterial activity against both community- and hospital-acquired MRSA. An in vitro post-antibiotic effect of 2.0–2.1 h is observed as well as a low spontaneous frequency of resistance, findings which support additional clinical evaluation of this compound for complicated skin and skin structure infections.

**P1103** Characterisation of resistance following serial passage of *Staphylococcus aureus* in the presence of the novel oxazolidinone TR-700 and linezolid

J. Locke*, K. Shaw (San Diego, US)

Objectives: To characterise the potential for *Staphylococcus aureus* to develop resistance over time to TR-700 (the active moiety of the novel oxazolidinone phosphate prodrug TR-701) and linezolid (LZD) through serial passage, and to elucidate the underlying resistance mechanisms.

Methods: *S. aureus* strains ATCC 29213 (MSSA), ATCC 33591 (MRSA), and CM-05 (cfr+, LZD-resistant MRSA (LMRSA)) were cultured at 37°C on Mueller-Hinton agar (MHA) or in liquid broth (MHB). MIC values were determined via microdilution in broth (MHB). No mutants were isolated at 8×MIC (2 mg/L) (frequency <2.9×10^-10). MICs of the mutants were similarly increased to pristinamycin and erythromycin A, but not to pefloxacin, chloramphenicol or tetracycline. No mutants of *S. aureus* AS5155 were isolated at concentrations of 2, 4, or 8×MIC (1, 2, and 4 μg/mL, respectively) (frequency <3.1×10^-10).

**Results:** Spontaneous mutation frequencies for MSSA 29213 and MRSA 33591 to TR-700 and LZD at 2×MIC were <1.2×10^-10 and <2.0×10^-10, respectively. These values are 16-fold lower than the corresponding LZD spontaneous mutation frequencies for both strains. No spontaneous mutants for LMRSA CM-05 were generated for either compound at 2×MIC. MIC values of TR-700 and LZD spontaneous mutants were 2 to 4-fold greater than the MSSA 29213 and MRSA 33591 wild type control MICs. MIC values for TR-700 were significantly lower than LZD for all of the mutant strains. The only 23S rRNA mutation detected for TR-700 was T2500A. Mutations for LZD included T2500A and G2447T. Some of the TR-700 and LZD-resistant mutants lower than LZD for all of the mutant strains. The only 23S rRNA mutations were determined through sequencing the domain V region of all 23S rRNA gene copies for each strain.

**Conclusions:** Spontaneous 23S rRNA mutations conferring resistance to TR-700 occur at an order of magnitude less frequently in *S. aureus* than those conferring resistance to LZD. Furthermore, for both of the 23S rRNA mutations reported here, TR-700 maintains a 4-fold or greater MIC advantage over LZD. Our analyses of these properties for TR-700 support the continued clinical development of TR-701.

**P1104** Characterisation of the novel oxazolidinone TR-700 and linezolid spontaneous mutation frequencies and resistance mechanisms in *Staphylococcus aureus*

J. Locke*, K. Shaw (San Diego, US)

Objectives: To compare the spontaneous mutation frequencies of 3 *Staphylococcus aureus* strains against TR-700 (the active moiety of the novel oxazolidinone phosphate prodrug TR-701) and linezolid (LZD), and to elucidate the underlying resistance mechanisms.

Methods: *S. aureus* strains ATCC 29213 (MSSA), ATCC 33591 (MRSA), and CM-05 (cfr+, LZD-resistant MRSA (LMRSA)) were cultured at 37°C on Mueller-Hinton agar (MHA) or in liquid broth (MHB). MIC values were determined via microdilution in accordance with CLSI guidelines. Spontaneous mutation frequencies were determined through plating of ~1×10^10 CFU on large-format (245×245 mm) MHA plates containing 2×MIC of TR-700 or LZD. 23S rRNA mutations were determined through sequencing the domain V region of all 23S rRNA gene copies for each strain.

**Results:** Spontaneous mutation frequencies for MSSA 29213 and MRSA 33591 to TR-700 and LZD at 2×MIC were <1.2×10^-10 and <2.0×10^-10, respectively. These values are 16-fold lower than the corresponding LZD spontaneous mutation frequencies for both strains. No spontaneous mutants for LMRSA CM-05 were generated for either compound at 2×MIC. MIC values of TR-700 and LZD spontaneous mutants were 2 to 4-fold greater than the MSSA 29213 and MRSA 33591 wild type control MICs. MIC values for TR-700 were significantly lower than LZD for all of the mutant strains. The only 23S rRNA mutation detected for TR-700 was T2500A. Mutations for LZD included G2447T and T2500A. Some of the TR-700 and LZD-resistant mutants did not have any mutations in the 23S rRNA domain V region and are undergoing further rRNA sequence analysis.

**Conclusions:** Spontaneous 23S rRNA mutations and the underlying resistance mechanisms are important parameters for evaluating the clinical utility of an antibiotic compound. We have demonstrated that spontaneous 23S rRNA mutations conferring resistance to TR-700 occur at an order of magnitude less frequently in *S. aureus* than those conferring resistance to LZD. Furthermore, for both of the 23S rRNA mutations reported here, TR-700 maintains a 4-fold or greater MIC advantage over LZD. Our analyses of these properties for TR-700 support the continued clinical development of TR-701.

**P1105** Enzymatic inhibition of *Streptococcus pneumoniae* PBP 2x transpeptidase activity by ceftaroline

A. Zerwasen, J.M. Firen*; A. Zapun (Liège, BE; Grenoble, FR)

**Background:** Cefartoline (CPT) is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Streptococcus pneumoniae* (MDRSP), as well as common Gram-negative pathogens. CPT is currently in phase 3 development. In order to understand the antipneumococcal activities of CPT, enzymatic inhibition studies were performed to examine the acylation of pneumococcal penicillin-binding protein 2x (PBP2x) by CPT.

**Methods:** This enzyme kinetic study was carried out with purified pneumococcal PBP2x from a penicillin-sensitive *S. pneumoniae* (PSSP) and PBP2x R6 isolate and a penicillin-resistant *S. pneumoniae* (PRSP) PBP2x 5204 isolate. The acylation is characterised by the second order rate constant k2/K. k2/K was determined by observing the competitive interaction between the reporter substrate, a thioester (S2d) and the inactivator CPT. Comparators, whose activity against PBP2x have been well characterised previously, included penicillin G (PEN) and cefotaxime (CTX).

**Results:** The inactivation of PBP2x by CPT was extremely rapid, making it impossible to study the kinetic parameters by classical methods. A k2/K-value of 106 M^-1 s^-1 was determined for PSSP by using the equation of a second-order reaction with equimolar concentrations of PBP2x and CPT. The k2/K-value for PRSP was >2300 M^-1 s^-1 for CPT, which was significantly higher than that of PEN (104 M^-1 s^-1) or CTX (85 M^-1 s^-1). The relative inhibitory activities of these drugs against PBP2x correlated with their MICs against the organisms.

**Conclusions:** CPT is a potent inhibitor of *S. pneumoniae* PBP2x and has excellent bactericidal activity against PRSP, in part through rapidly forming an inhibitory acyl enzyme intermediate with target PBP2x. CPT offers promise for the treatment of respiratory tract infections caused by drug-resistant pneumococci.
New antimicrobials

P1106 Efficacy of a new quinolone (UB-8902) in an experimental murine pneumonia model caused by Acinetobacter baumannii resistant to ciprofloxacin

Objective: The new quinolone UB-8902 derived from ciprofloxacin showed good in vitro activity, low toxicity parameters, and efficacy in a non-discriminative murine model of sepsis caused by Acinetobacter baumannii resistant to ciprofloxacin (ECCMID 08, poster n° P551). The aim of the present work is to determine its pharmacokinetic profile, and evaluate its efficacy against A. baumannii in a discriminative murine model of pneumonia.

Methods: We used two A. baumannii strains (Ab58 and Ab33). The antibiotics studied were ciprofloxacin (CIP), moxifloxacin (MOX), and the new generated quinolone UB-8902 (UB). Animals used were immunocompetent C57BL/6 female mice. Serum pharmacokinetic/pharmacodynamic (PK/PD) parameters [Cmax (mg/L), AUC (mg*h/L), T1/2 (h); AUC/MIC; T}\textsuperscript{0−24h} < 0.05\textsuperscript{<}< 0.05>\textsuperscript{vs} vs control group. Statistical analysis: ANOVA, post hoc test, and Chi-square tests.

Results: MICs (mg/L): Ab58, UB=0.03, CIP=0.25, MOX=0.016; Ab33, UB=0.5, CIP=8, MOX=1. PK parameters for UB, CIP and MOX, respectively, were: Cmax (mg/L), 7.91, 11.57, and 6.84; AUC (mg*h/L), 3.28, 8.79, and 3.56; T1/2 (h), 0.23, 0.27, and 0.34. PD parameters for Ab58 (UB, CIP, and MOX): AUC/MIC were 109.18, 35.16, and 222.71; T}\textsuperscript{0−24h} = 0\textsuperscript{0}<0>\textsuperscript{<} < 0.05 with control group. The MICs of UB, CIP, and MOX were 0.5, 8, and 1 mg/L, respectively, for the resistant ciprofloxacin strain.

Conclusions: The new quinolone UB-8902 is efficacious in an experimental pneumonia model caused by A. baumannii. This efficacy is also shown against the resistant ciprofloxacin strain.

P1107 Activity of PZ-601 (SMP-601) against Enterobacteriaceae with AmpC, ESBLs and carbapenemases

Background: PZ-601 (SMP-601) is a novel 1-\beta-methyl carbapenem, active vs. methicillin-resistant staphylococci and enterococci, including Enterococcus faecium, also vs. Enterobacteriaceae. We investigated how its anti-enterobacterial activity was affected by β-lactamases, alone or combined with porin loss.

Methods: AmpC expression mutants, transconjugants/transformants and clinical isolates were used, with MICs determined by the CLSI agar method.

Results: Among Enterobacter and Citrobacter freundii mutant series inducible, derepressed and basal AmpC were associated with MICs of 0.5–2 mg/L, 4–16 mg/L and 0.06–0.25 mg/L respectively, meaning that either inducible or (more so) derepressed AmpC expression gave some protection. Similar patterns arose for Serratia and Morganella spp., though the degree of protection was less. The chromosomal Class A β-lactamase of Proteus vulgaris did not protect, however expressed. None of the classical and extended-spectrum TEM, SHV or CTX-M enzymes transferred into Escherichia coli protected, with PZ-601 MICs consistently <1 mg/L for transconjugants. Among OXA enzyme, only OXA-3 protected, raising the PZ-601 MIC to 8 mg/L whilst not affecting MICs of other carbapenems. NMC-A, KPC and IMP carbapenemases conferred resistance, with MICs 16–128 mg/L. The modal MIC of PZ-601 for ESBL-positive E. coli was 0.5 mg/L (range 0.25–8 mg/L), whilst that for strains with high-level AmpC was 2 mg/L (range 1–16 mg/L). The mode for entrapenem-susceptible ESBL-positive Klebsiella spp. was 2 mg/L (range 0.25–8 mg/L), with little change for entrapenem-resistant produces lacking porins. The mode MIC for ESBL-positive Enterobacter spp. was 8 mg/L (range 1–32 mg/L); that for AmpC-deregpressed Enterobacter was 8 mg/L (range 4–16 mg/L) rising to 16 mg/L (range 16–24 mg/L) where porin loss and entrapenem resistance was also present.

Conclusions: PZ-601 seems highly stable to ESBLs, which were poor at conferring resistance even when combined with impermeability Resistance was seen in AmpC-producing Enterobacteriaceae, particularly Enterobacter spp. and was increased if AmpC expression was derepressed and coupled with impermeability. Carbapenemases conferred resistance.

P1108 Efficacy and safety of doripenem versus comparators in subjects with Acinetobacter baumannii: integrated analysis of six phase III clinical studies

Objectives: With some strains of A. baumannii resistant to nearly all antibacterials it is important to evaluate available agents for treatment of infections due to this pathogen. Doripenem (DORI) is a carbapenem with activity against Gram-negative bacteria, including A. baumannii. The goal of this analysis is to present the clinical effectiveness of DORI vs. comparators (COMP) in the treatment of infections associated with A. baumannii.

Methods: An analysis was conducted on a subset of subjects with A. baumannii isolated at study entry and included in the mITT population from 6 studies [complicated urinary tract infection (cUTI, 2 studies), complicated intra-abdominal infection (cIAI, 2 studies), nosocomial pneumonia (NP, 1 study) and ventilator-associated pneumonia (VAP, 1 study)]. Clinical success at test-of-cure (TOC) was determined for subjects by disease, and the pooled differences in success rates for DORI vs. COMP agents (imipenem [IMI], meropenem [MER], levofloxacin [LVX], piperacillin/tazobactam [P/TAZO]) across the studies also were determined.

Results: 44/1406 (3.1%) DORI-treated and 31/1043 (3.0%) COMP-treated mITT subjects had A. baumannii. At TOC, 61.4% (27/44) of DORI-treated subjects had clinical success vs. 35.5% (11/31) of COMP-treated subjects by disease, and the pooled differences in success rates for DORI vs. COMP agents (IMI, MER, LVX, P/TAZO) across the studies also were determined.

Conclusion: The pooled difference in clinical success rates favoured DORI regardless of comparator agent suggesting DORI may be an effective alternative for the treatment of infections caused by susceptible strains of A. baumannii.
**P1109** Doripenem clinical and microbiologic outcomes by baseline susceptibility for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from 5 phase 3 clinical trials


**Background:** Effective treatment options for infections caused by *P. aeruginosa* and *A. baumannii* are limited. A promising alternative to current agents could be doripenem (DOR), a new carbapenem approved for the treatment of adults with complicated intra-abdominal (cIAI) and urinary tract infections (cUTI) in the United States, and cIAI, cUTI, and nosocomial pneumonia (NP) in Europe.

**Methods:** Per-pathogen clinical cure (CC) and microbiologic cure (MC) rates for *P. aeruginosa* and *A. baumannii* from 5 phase 3 clinical trials were assessed. Rates for all isolates and for the subset susceptible to the study drug received were compared between DOR and pooled comparator agents in subjects who were microbiologically evaluable (ME) at the test of cure (TOC) visit as well as those in the microbiological modified intent-to-treat (mMITT) population.

**Results:** CC rates for all *P. aeruginosa* and *A. baumannii* isolates combined were significantly higher for DOR vs pooled comparators (84.2% vs 60.0%; 95% confidence interval [CI] 11.0−37.3) and for the subset of isolates with DOR minimum inhibitory concentrations (MICs) ≤4 µg/mL vs susceptible comparators (85.6% vs 67.6%; 95% CI 4.1−31.9). CC rates for all *P. aeruginosa* were also significantly higher for DOR vs pooled comparators (83.8% vs 60.3%; 95% CI 8.7−38.3) and for isolates with DOR MIC ≤4 µg/mL vs susceptible comparators (83.6% vs 66.7%; 95% CI 1.5−32.7). MC rates for all *P. aeruginosa* and *A. baumannii* combined were significantly higher for DOR vs comparators (81.2% vs 66.3%; 95% CI 1.7−28.1) and numerically higher for isolates with DOR MIC ≤4 µg/mL vs susceptible comparators (81.4% vs 66.7%; 95% CI 0.5−28.2). Parallel results occurred in the mMITT population.

**Conclusion:** DOR was clinically and microbiologically more effective than the pooled comparators in patients infected with *P. aeruginosa* and *A. baumannii* causing cIAI, cUTI, and NP even when the pathogens were susceptible to the comparator drugs received.

**P1111** Bactericidal effect of the novel siderophore monobactam BAL30072

M. Page *, C. Müller, B. Hofer, E. Desarbe (Basel, CH)

**Objective:** BAL30072 is a novel monobactam antibiotic with potent in-vitro activity against Gram-negative non-fermentative bacilli. We have investigated the in-vitro time-kill kinetics of BAL30072 using a selection of multi-resistant bacteria.

**Methods:** MICs were determined by standard broth micro-dilution methods. A preliminary MBC was determined by plating out clear wells from the MIC determination and counting colonies after 24 and 48 h incubation. Time-kill studies were done in standard growth medium with an inoculum of approximately 10^6 cfu/mL. Serial dilutions were taken at 0, 3, 6 and 24 h after inoculation.

**Results:** The MIC of BAL30072 was 1−4×MIC against most strains of Enterobacteriaceae and Acinetobacter spp. The MBC could be up to 16×MIC against some strains of *Pseudomonas aeruginosa* that were tested. Against the same strains the MBCs of ceftazidime and meropenem were 1−4×MIC for susceptible strains of Enterobacteriaceae and 4−32×MIC for susceptible strains of *Acinetobacter* spp. and *P. aeruginosa*. Time-kill analysis demonstrated that BAL30072 decreased the cfu count by 3 orders of magnitude within 24 h at 4×MIC against Enterobacteriaceae, Acinetobacter and at 8×MIC for *P. aeruginosa*. The kill kinetics were dependent on inoculum density and were faster for an inoculum of 5×10^7 cfu/mL than for an inoculum of 10^8 cfu/mL. The initial kill kinetics typically depended on the concentration of BAL30072.

**Conclusions:** BAL30072 has marked bactericidal activity against a range of multi-resistant Gram-negative bacteria, including carbapenem-resistant strains.

**P1112** In vitro activity of a new siderophore monobactam BAL30072 against ESBL-producing Enterobacteriaceae and clinical isolates of *Enterobacter cloacae*

K. Bowker *, T. Walsh, A.P. MacGowan (Bristol, Cardiff, UK)

**Objectives:** BAL30072 (BAL) is a new siderophore monobactam with in-vitro activity against many multiresistant aerobic Gram negative rods including *Acinetobacter* spp and *Pseudomonas aeruginosa*. In this study, we assessed the potency of BAL against clinical isolates of ESBL-producing Enterobacteriaceae and *Enterobacter cloacae* (Ent.cloacae) as wild type *Enterobacter* spp may have aztreonam MIC of >8 mg/L.

**Methods:** 148 strains were tested. 51 ESBL producers, *E. coli* n = 34; *K. pneumoniae* n = 10; Ent.aerogenes n = 7; and 97 clinical isolates of Ent.cloacae. Five antimicrobials were used: BAL; meropenem (MERO; ceftazidime (CTAZ); piperacillin-tazobactam (P/T); ceftazidime (CFEP). MICs were determined by CLSI methods but Muller-Hinton agar supplemented with 2.2 dimpyridyl was used for BAL to induce iron transport.

**Results:** MICs, range, MIC50, MIC90 (mg/L) are shown in the table.

<table>
<thead>
<tr>
<th>ESBL producers</th>
<th>BAL</th>
<th>MRO</th>
<th>CTZ</th>
<th>P/T</th>
<th>CFEP</th>
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<tbody>
<tr>
<td><em>E. coli</em> range 0.05−0.64 0.008−0.06 2−8.4 6.4 4.6 0.12−0.64</td>
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<td>MIC50 1</td>
<td>0.005</td>
<td>10</td>
<td>32</td>
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<tr>
<td>MIC90 2</td>
<td>0.015</td>
<td>32</td>
<td>64</td>
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<td><em>K. pneumoniae</em> range 0.06−0.64 0.015−0.06 2−8.4 6.4 4.6 0.12−8.4</td>
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<tr>
<td>MIC50 1</td>
<td>0.015</td>
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<td>MIC90 2</td>
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<tr>
<td><em>Ent. aerogenes</em> range 0.06−0.64 0.015−0.06 0.12−8.4 6.4 4.6 0.05−8.4</td>
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<td>MIC50 1</td>
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<td>MIC90 2</td>
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</table>

**Clinical strains**

| Ent.cloacae range 0.008−0.64 0.008−0.015 0.12−8.4 1−8.4 0.015−16 |
| MIC50 1 | 0.015 | 4 | 10 | 0.12 |
| MIC90 2 | 0.64 | >64 | 64 | 4 |

**Conclusions:** MERO was the most potent β-lactam against these ESBL producers, and Ent.cloacae. BAL shows in vitro activity against the majority of ESBL producers and also clinical strains of Ent.cloacae.
**P1113** Pharmacokinetics, pharmacodynamics and safety profile of a fully human IgM anti-Pseudomonas aeruginosa serotype O11 monoclonal antibody KBPA 101 in healthy volunteers

H. Lazar*, M.P. Horn, A.W. Zaucher, M.A. Imboden, P. Durrer, M. Seiberling, R. Pokorny, C. Hammer, A.B. Lang (Bern, Lausanne, Allschwil, Genexa, CH)

Objectives: To obtain pharmacokinetics, pharmacodynamics and safety data after first administration of KBPA 101, in humans.

Methods: In a double blind, dose escalation study, 32 subjects were randomised in 4 groups to receive a single intravenous infusion of KBPA 101: 0.1, 0.4, 1.2 or 4 mg/kg body weight (in each group 6 subjects received KBPA 101 and 1 placebo). Plasma samples for pharmacokinetics were taken pre-dose and at different times up to 14 days after start of dosing. Blood samples for analysis of antibodies to KBPA 101 were obtained at screening and on days 7 and 14. Blood samples for assessment of C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), interleukin-8 (IL-8), and total complement activity (TCA) were obtained at pre-dose, and at different times up to 48 hours after infusion.

Results: Plasma concentrations of KBPA 101 showed a mean maximum concentration of 1877, 7571, 24923 and 83197 ng/mL following doses of 0.1, 0.4, 1.2, and 4.0 mg/kg body weight, respectively. Mean elimination half life ranged from 70 to 95 hours. Mean volume of distribution was between 4.76 and 5.47 litres. Clearance ranged between 0.039 and 0.120 L/hr. KBPA 101 exhibited linear kinetics across all doses. No anti-KBPA antibodies were detected. No clinically significant variation was observed in CRP, TNF-α, IL-8, and TCA. No serious adverse events were observed and none of the subjects discontinued due to an adverse event. Eight out of 32 subjects reported nine adverse events (7 mild and 2 moderate). Seven subjects who received KBPA 101 had 7 adverse events (6 mild, 1 moderate) and one subject on placebo had 2 adverse events (1 mild, 1 moderate). Three subjects reported 3 adverse events (all mild) related toKBPA 101 (feeling of pressure in head, pressure at heart site and headache). All adverse events resolved without sequelae. There was no increase in the incidence of adverse events with increasing dose.

Conclusion: The fully human IgM anti-Pseudomonas aeruginosa monoclonal antibody KBPA 101 was well tolerated over the entire dose range, no serious adverse events were observed, no clinically significant variation was observed in any inflammatory markers and its pharmacokinetic profile was similar to a native IgM.

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**P1114** Developing DNA-based therapies to tackle antibiotic resistance

J. Moore*, M. McArthur, M.J. Bibb (Norwich, UK)

Background: With the prevalence of antibiotic resistance amongst pathogenic bacteria increasing, alternative therapies to combat bacterial infection are required. We are developing a novel approach to combat the spread of antibiotic resistance in pathogenic bacteria: Transcription Factor Decays (TFDs). TFDs are short stretches of DNA that contain the binding site for a targeted transcription factor; when introduced into cells in sufficient number TFDs sequester the transcription factors and so prevent them from binding their genomic targets within promoters, with a concomitant modification of gene expression. As such TFDs represent a universal genetic tool capable of altering prokaryotic phenotypes.

Objectives:
1. To develop this technology further so it can be used against a range of clinically relevant bacteria.
2. Development of the methods of transfection.
3. Identification of new TFD targets.

Methods: The effectiveness of the TFDs and transfection method assessed by bacterial growth rate TFDs created by PCR

Results:
1. *E. coli* grown with TFDs display reduced growth.
2. *S. aureus* grown with TFDs display reduced growth and in some cases viability was below detectable levels.

3. TFDs can be used to sensitising *M. smegmatis* to antibiotics.

Conclusions: As such TFDs represent a universal genetic tool capable of altering prokaryotic phenotypes which we have now applied to sensitising previously resistant pathogenic bacteria to antibiotics.

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**P1115** SASP: A novel antibacterial protein with potential to limit the spread of antibiotic resistance

S. Holme, A. Wilkinson, H. Fairhead* (Cambridge, UK)

Objectives: Antibiotic resistance spread by mobile genetic elements is of significant clinical importance. An antibacterial agent that can also inactivate such elements and prevent their spread between bacteria could provide profound clinical benefit. Novel antibacterial protein, SASP binds to all bacterial DNA in a non sequence-specific manner halting DNA replication and gene transcription. It is rapidly bactericidal. SASPject is a novel technology using modified bacteriophages to deliver SASP genes to target pathogens. SASPject PT1.2 delivers SASP genes to *S. aureus*, including MRSA. The ability of SASP to limit the transfer of plasmid-derived antibiotic resistance by rendering plasmid DNA unusable by other bacteria was assessed and compared to ciprofloxacin (Cip), a DNA gyrase inhibitor.

Methods:
1. The effect of purified SASP on the transfer of antibiotic resistance: *E. coli/S. aureus* shuttle plasmid pSM198 (ampicillin (Amp)/tetracycline (Tet) resistant) was incubated with purified SASP at ratios between 0.1 to 10.1 SASP:DNA. DNA from each reaction was precipitated and used to transform competent *S. aureus*. Transformants were enumerated after 16h incubation at 37°C on Luria-Bertani (LB) agar plates containing Tet (0.01 mg/ml).
2. The effect of PT1.2-delivered SASP vs Cip on the transfer of antibiotic resistance: A culture (3×10^6 cfu) of *S. aureus* carrying pSM198 was infected with 6×10^6 pfu PT1.2 or its Wild Type parental phage, or treated with Cip (1 or 4×MIC). After 45 min incubation, collected cells were lysed. Lysate (5 microlitres) was used to transform *E. coli*. Transformants were enumerated after 16h incubation at 37°C on LB agar plates containing Amp (0.1 mg/ml).

Results:
1. As SASP:DNA ratios increase, the number of transformed Tet resistant *S. aureus* cells decrease – up to 99% fewer compared with the control.
2. Lysate of PT1.2 infected cells produced >90% fewer Amp resistant *E. coli* transformants than lysate of parental phage, or Cip treated cells.

Conclusion: SASP’s unique antibacterial mode of action also gives it potential to reduce the spread of plasmid-derived antibiotic resistance from targeted pathogens, potentially generating significant clinical benefits.

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**P1116** In vitro susceptibility of *Campylobacter jejuni* to organic acids and monoacylglycerols

Z. Molatoka*, E. Skrivanova, M. Marounek, P. Brezina (Prague, Zlin, CZ)

Objective: Antimicrobial resistance among foodborne pathogens has been recognized as an important emerging public health problem. *Campylobacter jejuni* is one of the most common causes of foodborne diseases, moreover increasing its antimicrobial resistance has been reported. Therefore, it is important to develop means to control the transmission of *Campylobacter jejuni* from food to humans. Organic acids, used for decades as food and feed preservatives, continue to be the alternative of choice. The aim of the present study was to evaluate antimicrobial activity of 19 organic acids and two monoacylglycerols against *C. jejuni* by the SYBR Green based real-time PCR.

Methods: Susceptibility of *C. jejuni* CCM 6214 (ATCC 33560) to 19 organic acids (acetic, propionic, butyric, caproic, caprylic, caprylic, lauric, myristic, palmitic, stearic, oleic, sorbic, fumaric, succinic, benzoic, phenylacetic, lactic, malic and citric) and two monoacylglycerols (monocaprylin a monocaprin) was determined in cultures grown on...
A note on the effect of medium-chain fatty acids on intracellular activity of anidulafungin against Candida species. The highest prevalence was found in poultry, followed by pork by handling or consumption of contaminated water and food of animal origin. The clinical significance of these organisms to humans is limited. However, three species, A. butzleri, A. cryaerophilus and A. skirrowii have been found to be associated with human diarrheal illness. A. butzleri is the fourth most common Campylobacter-like organism isolated from the stool of human patients in Belgium and France. The association of Arcobacter spp. with human gastroenteric illness has also been reported in other countries. Infection may occur by handling or consumption of contaminated water and food of animal origin. The highest prevalence was found in poultry, followed by pork ad beef. The study aims to evaluate the effect of medium chain fatty acids on three predominant arcobacters, A. butzleri, A. cryaerophilus and A. skirrowii.

Methods: Antimicrobial activity of medium chain fatty acids (C6–C12) was tested in vitro by the real-time PCR method, using specific primers. Data were analyzed by the comparative Ct method. Positive control (untreated cultures of arcobacters) was used as the calibrator to express the relative fold-induction (2–ΔCt) for each sample.

Results: All arcobacters showed a very high sensitivity to all medium chain fatty acids tested. The IC50 values ranged from 0.1 to 1 mg/mL. Antimicrobial activity of organic acids tested was pH-dependent, being more pronounced at lower pH. The results of the present study show that antimicrobial activity of other organic acid against C. jejuni is variable and supplementation of feeds with some acids would not present any protection. Conclusion: Obtained results demonstrate the potential use of medium-chain fatty acids against Campylobacter jejuni at concentration feasible in vivo.

Acknowledgments: This study was supported by the Grant Agency of the Czech Republic (grant no. 525/08/I0606) and by the Ministry of Agriculture of the Czech Republic (project MZe 0002701404).

Methods: Sixteen new oxepins derivatives were synthesized in a several stages synthesis and the new compounds were characterised by their physicochemical properties and the chemical structures and purity were confirmed by elemental analysis and spectral studies (IR, 1H-NMR, 13C-NMR). The original compounds were solubilised in dimethylsulfoxide (DMSO) and screened for their in vitro antifungal activity against Gram-positive, Gram-negative bacteria and fungal strains, using both reference and clinical, multidrug resistant strains, using the qualitative adapted diffusion method and the quantitative assay of the antimicrobial activity performed by nutrient broth microdilution method in order to establish the minimal inhibitory concentration. The subinhibitory concentrations of the tested substances were investigated for their influence on the adherence capacity to the cellular substrate represented by HeLa cells and to inhibit substrata quantified by slime test and on the expression of soluble enzymatic virulence factors (haemolysins and other pore-forming toxins, proteases activity; DNA-se and siderophores production).

Results: Our results showed that the new compounds did not exhibit significant antimicrobial activity (MIC values >1 mg/mL). In exchange, they interfered with the expression of different virulence factors implicated in the pathogenicity of these opportunistic strains. All analyzed compounds decreased the ability of the tested microbial strains to adhere both to the cellular and inert substrata and also induced changes in the adherence patterns. Concerning the soluble virulence factors, the tested compounds induced decreases of various degrees (%) in the expression of haemolysins (28.5–86%), lipase (100–28.5%), lecitinase (17–67%), gelatinase (0–71.5%), caseinase (0–45%), DN-ase (0–53%) and siderophores production (13–100%). Conclusion: The present study proved that the new oxepins, in the absence of a significant antimicrobial activity, attenuated the virulence of different microbial strains, by inhibiting the expression of adhesion molecules and secretion of soluble, enzymatic factors thus altering the success of these pathogens in the colonisation of a sensitive host and the development of an infectious process.

Antimicrobial and anti-pathogenic features of some new oxepins


Objectives: The aim of the present study was to evaluate some newly synthesized new oxepins for their antimicrobial and anti-pathogenic features, respectively.
Candida albicans (susceptible and resistant to fluconazole strains) and Candida krusei inhuman polymorphonuclear leukocytes (PMNs) has been evaluated. Methods: C. albicans (two isogenic strains; fluconazole susceptible and resistant) and C. krusei were used for killing assays. Susceptibility studies were determined by microdilution according to CLSI, M27-A2. To evaluated the intracellular activity, PMNs and osponised yeasts (PMN/yeast; 1/10) were incubated for 30 min at 37°C. Extracellular yeasts were removed by differential centrifugation and PMN containing intracellular yeasts are incubated in RPMI for three hours in the presence of different extracellular concentrations of anidulafungin. Afterwards, cells are disrupted by osmotic shock and intracellular survival is determined by pour plating method (CFU counted onto Sabouraud Agar). The data were expressed as percentages of Candida surviving compared with levels in controls (without antifungal agent).

Results: At all extracellular concentrations evaluated (1, 5 and 10 mg/L), anidulafungin reduced significantly the surviving intracellular Candida krusei (percentage of surviving Candida compared to the control: 52± 12, 38±5 and 32±8 respectively). At extracellular concentrations of 5 and 10 mg/L, anidulafungin also showed significant intracellular activity against both strains of Candida albicans (fluconazole susceptible and resistant).

Conclusions: At extracellular concentrations higher than 1 mg/L, anidulafungin showed significant intracellular activity against Candida albicans and Candida krusei.

P1121 Activity of N-chlorotaurine against protozoa
U. Fünkranz, J. Walochnik, M. Nagl* (Vienna, Innsbruck, AT)

Objectives: N-chlorotaurine (NCT), a long-lived oxidant produced by human leukocytes, can be synthesized chemically as sodium salt and is presently investigated as an antiseptic. It is a mild active chlorine compound and very well tolerated at different body sites. The aim of the present study was to investigate its activity against protozoa which might come into question for topical treatment.

Methods: Promastigotes and amastigotes of Leishmania infantum and L. donovani, Trichomonas vaginalis, and trophozoites and cysts of Acanthamoeba were suspended in NCT solutions, and after different incubation times the killing of parasites was evaluated by trypan blue staining and microscopy.

Results: Viability of all tested protozoa was reduced by NCT. Times needed for complete killing by a concentration 1% NCT (55 mM) were approximately 2 h for leishmaniae, 1 h for acanthamoebae, and 15 min for trichomonads. Inactivation of parasites was still observed at tenfold lower NCT-concentrations. A delay of excystation of amoebae could also be detected. However, cysts could only be inactivated completely by addition of ammonium chloride to NCT, which enhanced the activity against all tested parasites significantly.

Conclusion: NCT demonstrated broad-spectrum activity against protozoa and it may be considered for topical treatment of such infections. The booster effect by ammonium chloride can be explained by formation of monochloramine which penetrates pathogens better because of its higher lipophilicity.

P1122 Drug inhibition of HDAC activity: effect on the parasite Toxoplasma gondii and chemotherapy perspectives
D. Maubon, A. Bougour, Y.S. Wong, M.A. Hakimi, H. Pelloux*
(Grenoble, FR)

Toxoplasmosis is one of the most common parasitic diseases. The intracellular parasite T. gondii belongs to the Apicomplex family, as Plasmodium species, with which it shares lots of molecular similarities. T. gondii life cycle is complex and characterised by the interconversion phenomenon, which is the ability of the parasite to differentiate from a tachyzoite form to a cystic structure. Cysts are mostly asymptomatic in healthy people, but if reactivating, could be potentially life threatening in immunocompromised patients. It has been established that the epigenetic machinery of T. gondii is a major tool in the control of gene transcription and more precisely that acetylation of histones plays a substantial role in parasite interconversion.

Objectives: As a tool to clarify the role of epigenetic mechanisms in T. gondii, we used histone deacetylase inhibitors (HDACi) and observed the phenotypic and molecular consequences on the parasite. Potential therapeutic effect, on both tachyzoites and cyst forms was also evaluated.

Methods: In vitro studies were conducted with different T. gondii strains. Different HDACi belonging to the hydroxamic family (TSA, Scriptaid) or to the tetrapeptid cyclic family (Apicidin, DrugA, HC Toxin) were tested. Other drugs were also newly synthesized to further understand the mechanism of action and to optimise the anti-parasitic effect. Ex vivo cysts were also treated with DrugA, than injected in mice to study their infective power.

Results: Interfering on histone deacetylase(s) dramatically changed parasite phenotype, pushing it through bradyzoite conversion but also interrupting its proliferation. Only drugs belonging to the cyclic tetrapeptide family of HDACi showed significant efficiency without disrupting human host cells. Ex vivo cysts treated for 7 days with DrugA appeared morphologically normal, but were unable to infect mice as serology remained negative and as no cyst were detected in mice brains 6 weeks after infection.

Conclusions: Using HDACi as a chemical knock-out of hdac(s) on T. gondii, made us sight the importance of histone acetylation and epigenetic control of gene transcription in the natural course of parasite being. It also brought consistent results in growth inhibition of tachyzoites and in blocking the infective power of cysts, which are necessary to disease transmission. These results are promising in finding new therapeutic agents or molecular targets in toxoplasmosis and other apicomplex infection.

P1123 Mechanism of action of Melaleuca alternifolia (tea tree) oil on influenza A/PR/8 virus replication
B. Bissignano*, R. Timpanaro, A. Garreox, A. Sisula, G. Tempera, A. Castro (Catania, IT)

Objectives: Our previous study demonstrated that the Melaleuca alternifolia (Tea Tree) Oil (TTO) had an interesting antiviral activity against Influenza A/PR/8 virus subtype H1N1 in MDCK cells. When we tested TTO and some of its components we found the TTO, the terpinen-4-ol, the terpinolene, the alpha-terpinolene to have an inhibitory effect on Influenza virus replication at doses below the cytotoxic dose; the terpinene-4-ol was the main active component.

Studies on time of addition experiments suggested that the TTO exerts an interference with an early step of the viral replicative cycle of Influenza virus. The aim of this study was to investigate the effect of TTO on viral attachment and on acidification of endosomal/lysosomal compartments of living cultured cells.

Methods: The inhibition of attachment was studied by the infective centre assay and the effects of the TTO on acidification of lysosomes were tested by vital staining with acridine orange using Bafilomycin A1 as positive control. MDCK culture cells, stained with acridine orange, were examined by a fluorescence light microscopy or quantified in RFUs (relative fluorescence units) using a fluorimeter Wallac Victor 2 multilabel counter.

Results: The influence of the TTO on the virus adsorption step, studied by the infective centre assays, indicated that TTO did not interfere with cellular attachment of virus. When MDCK cells were stained with acridine orange, nuclei and the cytoplasm showed green fluorescence, whereas orange fluorescence was observed in a granular pattern in the cytoplasm, due to acidified lysosomes. Treatment of cells with 0.01% (v/v) and 0.005% (v/v) of TTO at 37°C for 2 h before staining caused complete disappearance of the orange fluorescence, whereas the green fluorescence remained. This results was confirmed by measuring the fluorescence intensity by fluorometry, indicating that TTO clearly inhibited acridine orange accumulation in acid cytoplasmic vesicles.
**Bio-informatic prediction of herpes simplex virus type 1 latency-associated transcript microRNA and their mRNA neurotransmitter targets**

W Kwanhian, W Namwat, V Lulitanond* (Khon Kaen, TH)

In herpes simplex virus type 1 (HSV-1) latent infection, no viral gene is transcribed, except the latency-associated transcript (LAT) gene. Although LAT is able to transcribe to mRNA and accumulates at high copy number in the nuclei of latently infected cells, its protein product is not found. Previous studies showed that HSV-1 LAT expression in sensory neuron affected the level of certain neuropeptide expressions, especially substance P (SP) and calcitonin-gene related peptides (CGRP) genes, but its mechanism is still unclear. Recently, HSV-1 LAT derived microRNAs (miRNAs) have been proposed for the regulation of both viral and host genes expression. For these reasons, we speculate that HSV-1 LAT-miRNA might regulate SP and CGRP expression by using its mRNA property.

**Objective:** To predict miRNAs generated from HSV-1 LAT mRNA precursor and to characterise their mRNA neurotransmitter targets by bioinformatic approaches.

**Methods:** The web-based programs, Bayes-SVM-MiRNA web server version 1 (http://wotan.wistarr.upenn.edu/BayesSVMMiRNAfind/), was used to predict miRNAs generated from HSV-1 LAT mRNA and the RNAhybrid program (http://bibiserv.techfak.uni-bielefeld.de/rahybrid/submission.html) was used to locate the binding site of SP and CGRP mRNA targets and their binding activities.

**Results:** Two HSV-1 LAT miRNA (V1-miRNA and V2-LAT-miRNA) with the size of 21 nucleotides were predicted from Bayes-SVM-MiRNA program. After defining targets of HSV-1 LAT miRNAs in Tac 1 (SP gene) and CGRP miRNAs to RNA hybrid program, it was found that V1-miRNA and V2-LAT-miRNA are able to bind to internal site of target mRNAs at highest negative MFE (minimum free energy) of −28.4 and −32.4 kcal/mol, respectively.

**Conclusion:** In order to save time and resources before implementation of any experimental works, we have used the web-based program to predict HSV-1 LAT miRNAs and their neurotransmitter targets. The results are encouraging for further verification by experimental approaches in order to elucidate the biological role of HSV-1 LAT.

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**In vitro activity of five antimicrobial peptides against colistin-susceptible and resistant Acinetobacter spp.**

X. Vila*, S. Marti, E. Giralt, J. Vila (Barcelona, ES)

**Objective:** To test the activity of five antimicrobial peptides (AMPs) against *Acinetobacter baumannii*, *Acinetobacter* genospecies 3 and *Acinetobacter* genospecies 13, which were susceptible and resistant to colistin.

**Methods:** Three *Acinetobacter* clinical isolates, belonging to *A. baumannii*, *Acinetobacter*. genospecies 3 and *Acinetobacter*. genospecies 13 were chosen to perform this study. The *A. baumannii* ATCC 19606 was also included. Colistin-resistant mutants of the above mentioned strains were selected by serial passages from each strain in subinhibitory concentrations of colistin. The MICs of magainin II, cepoazen P1, buforin, indolicidin, a-defensin for colistin-susceptible and resistant *Acinetobacter* spp. were determined with a microdilution assay using both cation-supplemented and unsupplemented Muller-Hinton broth.

**Results:** The results of the MICs using both media were similar with only slight differences. All colistin-resistant *Acinetobacter* spp. mutants showed a MIC of colistin of 256 mg/L. The AMPs could be distributed in three groups according to their activity: i. Magainin II, a-defensin, and buforin did not show activity against colistin-susceptible and -resistant *Acinetobacter* spp. (MIC > 32 mg/L for both colistin-susceptible and -resistant *Acinetobacter* spp.). ii. Cepoazen P1 showed similar activity as colistin (MIC of 0.5 mg/L for the susceptible strain and of >32 mg/L for the resistant strain) and iii. Indolicidin showed a good activity for both colistin-susceptible and -resistant Acinetobacter spp. (MICs of 1–2 mg/ml for both susceptible and resistant Acinetobacter spp.).

**Conclusion:** Indolicidin shows good activity against colistin-susceptible and -resistant *Acinetobacter* species, suggesting a different mechanism of resistance than that for colistin. This peptide may be a potential drug for treatment of panresistant *A. baumannii* infections.
Update on pneumococcal vaccines: 7, 10, 13 or 23?

**P1128**

Assessment of all-cause pneumonia admissions before introduction of the pneumococcal conjugate vaccine in Switzerland

B.Y.Betsch*, K. Mühlmann (Berne, CH)

Objective: To describe the baseline epidemiology of hospitalised pneumonia before the introduction of the 7-valent pneumococcal conjugate vaccine in Switzerland in 2006.

Methods: National hospitalisation data were obtained from the Federal Institute of Statistics for the years 1998–2006 including the primary diagnosis (first-listed; International Classification of Diseases), up to 7 additional diagnoses, and other parameters characterising hospitalisation. Community acquired pneumonia (CAP) was defined by a primary diagnosis of pneumonia or meningitis/Septicaemia plus a code for pneumonia. Pneumococcal CAP (SpnCAP) was defined as CAP with a pneumococcal disease code. Hospitalisation rates for CAP and SpnCAP were calculated by segmented regression analysis.

Results: There were 122,572 hospitalisations for CAP (annual average 15,322). SpnCAP was coded in 5.1% of CAP. CAP hospitalisation rates showed a rising trend between 1998 and 2001 probably due to a reporting bias. Thereafter, annual CAP (SpnCAP) rates per 105 populations were stable at an average of 425 (21). Rates varied by age group with highest rates among the <2 years olds 362 (14) and the >80 years olds 1525 (64). Males predominated (57%) especially in the <2 years olds (58%), and the elderly (60%). Ethnicity was Swiss in 84% of CAP cases, but this proportion was lower for <2 years olds (73%) and increased with age to reach 90% in the elderly. Average hospital stay was 13 days, but stay was shortest for the younger age groups and increased with age from 6.1 days in the <2 years olds to 17 days in the >80 years olds. Case fatality rate was 7.4% overall with most (88%) fatal cases occurring in the elderly. Admission to intensive care treatment was needed in 6.2% of CAP.

Conclusion: Data for the pre-vaccine years 2002 to 2005 serve as baseline for evaluating the impact of conjugated pneumococcal vaccines.

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**P1129**

Invasive pneumococcal disease among adults in Germany after the start of the National Immunisation Programme for the 7-valent pneumococcal conjugate vaccine in children

M. van der Linden, R.R. Reinert, M. Imöhl* (Aachen, DE; Paris, FR)

Objectives: *Streptococcus pneumoniae* is a leading cause of pneumonia, sepsis and meningitis in Germany and disproportionately affects young children and the elderly. In July 2006 a general recommendation the vaccination with a 7-valent pneumococcal conjugate vaccine (PCV7) for all children up to the age of 24 months was made by the German Health authorities and the vaccination program was started in January 2007 in all federal countries with the exception of Saxony where vaccination started one year earlier. In this paper we present the effects of routine vaccination of young children with PCV7 on the rates of IPD in the adult population (indirect or herd immunity effect).

Methods: The National Reference Center for Streptococci has monitored the epidemiology of invasive pneumococcal disease (IPD) in adults in Germany since 1992. Cases of IPD in adults are reported by a laboratory-based surveillance system including 265 laboratories throughout Germany. For three federal states (North Rhine-Westphalia, since 2001, Saxony and Bavaria, since 2006) a population-based surveillance has been conducted. The present analysis includes cases from 2002 to 2009. In January 2007 a nationwide web-based surveillance system was introduced. Species confirmation was done by optochin testing and bile solubility testing. All isolates were serotyped using the Neufeld Quellung reaction.

Results: Due to enhanced surveillance the number of reported cases increased from 421 in the pneumococcal season 2003–2004 to 1761 in 2007–2008. Since reporting of IPD is not mandatory in Germany the calculation of incidences is not possible. However, a reduction of the percentage of IPD cases caused by vaccine serotypes should indicate a vaccination effect. From 2002 to 2006 the percentage of vaccine type IPD in adults in Germany has varied between 42.9% and 48.5%. In 2007–2008 this percentage has dropped to 33.4. In the three populations based studies we made similar observations. The 13-valent pneumococcal conjugate vaccine (in development) would have covered 72.9% of IPD in adults in 2007–2008 in Germany.

Conclusions: The introduction of routine childhood immunisation with PCV7 in Germany has led to a strong reduction of IPD in children in Germany. The results presented here show a reduction in the percentage of IPD cases in adults caused by vaccine serotypes. This might indicate a herd-immunity effect.

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**P1130**

Effectiveness of heptavalent pneumococcal conjugate vaccination on invasive pneumococcal disease one year after the introduction in the Danish childhood vaccination programme


Objective: On 1 October 2007, the heptavalent pneumococcal conjugate vaccine (PCV7) was introduced in the Danish childhood vaccination program. Vaccination was offered to all children born after 1 April 2006, at 3, 5, and 12 months of age (mo.) (2+1 schedule). A catch-up program of two doses was offered to children between 12–17 mo. We evaluated the effectiveness of PCV7 on invasive pneumococcal disease (IPD) one year after PCV7 introduction.

Methods: Prospective cohort study including nation-wide laboratory surveillance data on IPD and data on PCV7 coverage from the Danish Childhood Vaccination Registry. The pre-PCV7 period was defined as 2000–2007. The effectiveness of PCV7 was estimated for 2008 based on IPD data from 1 January to 15 December 2008. PCV7 coverage was estimated per 1 June 2008. For children aged 0–2 mo. at PCV7 implementation, 94% had received at least one dose and 74% at least
two doses. In the catch-up program, 86% of children between 3–14 mo. and 57% between 15–16 mo. received at least one dose. **Results:** In 2008, 840 IPD cases were registered vs. an annual average of 1048 cases in the pre-PCV7 period. 90% were bacteremia cases. The overall incidence of IPD declined from a mean of 19.4 to 15.3 per 100,000 comparing pre- and post-PCV7. In children <2 years, the mean incidence declined significantly from 54 to 23 per 100,000 in the pre- and post-PCV7 (p < 0.005). In children <2y, the mean incidence of IPD caused by vaccine serotypes decreased from 36 to 8 per 100,000. The most prevalent serotype in post-PCV7 was 7F (32%), all other serotypes accounted for less than 6% of cases. In pre-PCV7 period, the most prevalent serotypes were 14 (21%), 6B (20%), 7F (9%), 6A (8%), 19F and 23F (7%). In children between 2–5 y, the overall incidence slightly increased from 8.6 to 11.8 per 100,000. The incidence tended to decline in all other age groups: from 2.5 to 1.2 per 100,000 in persons between 5–18 y, from 7 to 5.8 per 100,000 in persons between 18–50 y, from 23.4 to 17.5 per 100,000 in persons between 50–64 y, and from 65.4 to 52.9 per 100,000 in persons older than 65 y comparing pre- and post-PCV7.

**Conclusion:** After the universal introduction of PCV7 in Denmark, we observed a decline in the overall incidence of IPD that was statistically significant in children younger than 2 years. In children younger than 2 years, serotype 7F was the dominant serotype in the post-PCV7 period. PCV7 coverage in children younger than 2 years was high.

**P1131** Serotype distribution of *Streptococcus pneumoniae* isolated from patients with invasive pneumococcal disease

W. Rosingh, E. Canton, G. Fagundez, F. Gonzalez, M. Gobernado *on behalf of the MIVA Network*

**Objective:** To describe the serotype distribution of *S. pneumoniae* associated with invasive pneumococcal disease in patients in the Valencia Community (5.9 million inhabitants), Spain, after introduction of the 7-valent pneumococcal conjugate vaccine (PCV7).

**Methods:** We serotyped *S. pneumoniae* isolated from blood, spinal fluid (CSF) and sterile liquid cultures collected during the year 2007 in patients who were diagnosed with invasive pneumococcal disease in any of the 19 participating hospitals. Serotyping was performed by antiserum agglutination (Denka Seiken, Tokyo Japan). If necessary more detailed serotyping was done by the Quelling reaction (Staten Serum Institute).

**Results:** We serotyped 363 isolates of *S. pneumoniae* from 363 patients (blood cultures, CSF and sterile fluids). The serotypes in order of frequency were: 19A (10.8%), 1 (10.8%), 14 (10.2%), 8 (8.6%), 7 (7.7%), 3 (6.9%), 4 (4.4%), 7F (3.6%), 6A (3.9%), 22F (2%). These serotypes represent a total of 69.9% of the total number of isolates that were serotyped. The other serotypes counted for percentages of less than 2.5%.

**Conclusions:** Of the serotypes isolated in this study, only 21.5% are included in the PCV7-vaccine. Continued surveillance is needed to guide development of future formulations of conjugate vaccines and to monitor the effects of continued vaccine use.

**PS:** Data used from the Surveillance Network of the Valencía Community (MIVA network). Public Health Department, Conselleria de Sanitat

**P1132** Clinical and economic impact of a 10-valent pneumococcal (Pnc)-non-typable *Haemophilus influenzae* (NTHi)-protein D conjugate vaccine (PHiD-CV) on the overall disease burden in Finland

S. Bergias *°, S. Torsvinen, T. Puimalainen (Espoo, FI)

**Objective:** To estimate the total impact of infant vaccination with a 7-valent pneumococcal vaccine (PCV-7), a 10-valent PHiD-CV or a no-vaccination strategy across all ages in Finland.

**Methods:** The total vaccine impact is assessed with a 1-year, cross-sectional, overall population-based model at vaccine steady-state condition selecting local epidemiological and unit cost data. Direct outcomes associated with invasive diseases (ID) such as meningitis and bacteremia, with community-acquired pneumonia (CAP) and with acute otitis media (AOM). Indirect vaccine effect of herd protection (HP) across the whole population, cross-protection and serotype replacement in IPD are also evaluated with the model. To obtain Finnish-specific vaccine effect results in IPD, local serotype distribution in ID of *Streptococcus pneumoniae* is multiplied by the serotype specific vaccine efficacy (VE). For CAP, an average vaccine efficacy for hospitalised and ambulatory pneumonia is used. Total VE in AOM is estimated as VE against vaccine serotypes, non-vaccine serotypes and NTHi. Vaccine cost is assumed to be equivalent for both vaccines.

**Results:** Compared with no-vaccination PHiD-CV is predicted to reduce IPD by 350 cases, pneumonia by 400 cases, tympanostomies by 7200 events and otitis by 77800 GP visits per year across the whole population once vaccine steady-state has been reached. Compared with PCV-7, PHiD-CV is predicted to prevent additionally 60 IPD cases, 65 pneumonias, 4300 tympanostomies and 45800 otitis GP visits per year. PHiD-CV vaccination is expected to result in cost savings compared with PCV-7 (10M€ in direct costs and 4M€ in productivity costs). Sensitivity analysis shows that the VE against tympanostomy and the cause of AOM have the greatest impact on the cost results. Using probabilistic sensitivity analysis the results indicate that vaccination with PHiD-CV is reaching cost savings compared with PCV-7 in 97% of the iterations.

**Conclusion:** Pnc and NTHi cause a significant disease burden in Finland. Especially the societal costs for pneumonia and otitis media are substantial. However this disease burden can be reduced with pneumococcal vaccination. PHiD-CV vaccination is expected to lead to cost-savings compared with PCV-7 when the vaccination cost is the same. If HP occurs in pneumonia cost-savings with the PHiD-CV vaccine are projected to be even greater.

**P1133** Serologic responses to revaccination with 23-valent pneumococcal polysaccharide vaccine among patients with HIV infection who received highly active antiretroviral therapy


**Background:** Revaccination with pneumococcal polysaccharide vaccine (PPV) is recommended to HIV-infected patients who are vaccinated five years earlier. Data of serologic responses to revaccination with 23-valent PPV among HIV-infected patients who continued to receive highly active antiretroviral therapy (HAART) with favourable immunologic and virologic responses are sparse.

**Methods:** Antibody responses to three pneumococcal capsular polysaccharides (serotypes 14, 19F, and 23F) that are prevalent in Taiwan were assessed among 129 HIV-infected patients who received revaccination with 23-valent PPV following primary vaccination using 23-valent PPV 5 years earlier. Three groups of patients were included according to baseline CD4+ counts when primary vaccination was administered: group 1, CD4+ <200 cells/μL (n = 55); group 2, CD4+, 200 to 349 cells/μL (n = 37); and group 3, CD4+ >350 cells/μL (n = 37). The proportions of responders who achieved increases of antibody titers
Antibody response to pneumococcal conjugate vaccination

Outpatient-based pneumococcal vaccine campaign and 8 weeks after the first application patients were vaccinated with pneumococcal conjugate vaccine Prevenar® (Wyeth Pharma, GmbH).

In the heart transplant (HTX) group, all patients received a relevant increase in antibody titers measured at week 16. In week 8 patients were vaccinated with PCV. However, the only difference to the LTX group would be followed by a transient appearance of activated antigen-specific effector lymphocytes in the circulation. The homing receptors (HR) enabling homing of circulating lymphocytes in the lung tissue are not yet known.

Methods: In the present study, patients with pneumococcal pneumonia and healthy volunteers immunised with pneumococcal polysaccharide vaccine were studied for circulating Streptococcus pneumoniae-specific antibody-secreting cells (ASC) appearing in the circulation. These cells were characterised for secretion of pathogen-specific antibodies using ELISPOT.

Results: In the pneumonia patients, the number of pathogen-specific ASC was 182/106 PBMC with IgG and in vaccinees 528/106 PBMC with IgA as the predominating Ig-isotype.

Conclusions: The present study shows that in pneumonia in humans pathogen-specific ASC appear in the circulation in humans, confirming the circulation of pulmonary lymphocytes in humans. The data also show differences in the in the Ig-distribution of these cells after natural infection and pneumococcal vaccination.

**Conclusion:** The level of perception and immunisation rate for the pneumococcal disease were quite low in South Korea.

**Circulating Streptococcus pneumoniae-specific effector B cells in humans in pneumococcal pneumonia and after pneumococcal polysaccharide vaccination**


**Objectives:** Streptococcus pneumoniae is the leading cause of pneumonia in all age groups. Pneumococcal vaccines are known to confer protection against an invasive pneumococcal infection. The protective immune mechanisms in lower respiratory tract are poorly characterised. Antigen encounter at mucosal sites is generally regarded to be followed by circulation of activated lymphocytes via lymphatics and blood back to the mucosal sites. Similarly, it has been suggested that antigen encounter in the lung would be followed by a transient appearance of activated antigen-specific effector lymphocytes in the circulation. The homing receptors (HR) enabling homing of circulating lymphocytes in the lung tissue are not yet known.

Methods: In the present study, patients with pneumococcal pneumonia and healthy volunteers immunised with pneumococcal polysaccharide vaccine were studied for circulating Streptococcus pneumoniae-specific antibody-secreting cells (ASC) appearing in the circulation. These cells were characterised for secretion of pathogen-specific antibodies using ELISPOT.

Results: In the pneumonia patients, the number of pathogen-specific ASC was 182/106 PBMC with IgG and in vaccinees 528/106 PBMC with IgA as the predominating Ig-isotype.

Conclusions: The present study shows that in pneumonia in humans pathogen-specific ASC appear in the circulation in humans, confirming the circulation of pulmonary lymphocytes in humans. The data also show differences in the in the Ig-distribution of these cells after natural infection and pneumococcal vaccination.

**Outpatient-based pneumococcal vaccine campaign and survey on perceptions about pneumococcal vaccination in patients and doctors**


**Background:** Despite the high morbidity and mortality of invasive pneumococcal diseases, vaccination rates have generally remained suboptimal around the world. In South Korea, only 0.6% of high-risk patients replied that they were encouraged to get the pneumococcal vaccine according to the nation-wide survey.

Methods: A cross sectional, community-based survey conducted to assess perceptions about pneumococcal vaccine at a local public health centre. In a tertiary teaching hospital, outpatient-based pneumococcal vaccine campaign was performed for the elderly and individuals with chronic medical conditions from May to July of 2007. Pneumococcal vaccine coverage rate among those high risk outpatients was assessed, and questionnaires were administered to assess perceptions on pneumococcal vaccination for the medical doctors and patients at pre- and post-campaign periods.

Results: Community based survey revealed that only 38 (7.6%) of 500 respondents have been informed of pneumococcal vaccine ever before, and none of them was vaccinated previously. When it came to the coverage rates of pneumococcal vaccine before and after the hospital campaign, annual rate was increased from 3.39% to 5.91%. The increments of pneumococcal vaccine coverage rate were statistically significant in patients with either chronic lung disease or chronic renal disease, while those among patients with diabetes, malignancy or chronic liver diseases were unremarkable. The most common reason for vaccination was “doctor’s advice” (53.3%). As for the interrupting factors of vaccination, about 75% of high risk patients were not aware of pneumococcal vaccine itself, and that was the most important barrier to vaccination. Secondly, doctor’s negative attitude was another important cause of non-vaccination.

Conclusions: In conclusion, the levels of perception and immunisation rate for the pneumococcal disease were quite low in South Korea.
Annual outpatient-based campaign around early influenza season would be efficient to improve pneumococcal vaccine coverage rate. Doctor's advice was the most important encouraging factor of vaccination, but government and health department should make efforts to improve patients’ perceptions on pneumococcal disease and vaccination.

Hand hygiene

**Objective:** 20,000 hand-hygiene observations on 60 wards in 16 acute trusts: dispelling hand-hygiene myths? Or reporting a national change in practice?


**Background:** Hand hygiene observation (HHO) is the gold-standard for measuring hand-hygiene compliance (HHC). Although published datasets are often small, past literature shows that compliance is poor (25–40%), is worse pre-patient-contact, in high risk clinical activities and falls as the number of hand-hygiene opportunities rises, and staffing levels fall. That of doctors is worse than nurses’.

**Objective:** Given the recent emphasis on hand-hygiene in the NHS, we re-examined these findings during the baseline phase of a national stepped wedge randomised controlled trial of an intervention to improve hand-hygiene, the FIT study.

**Methods:** From October 2006-April 2008, 670 hours of HHO (19755 observations) were undertaken an hour at a time, 6-weekly, on 60 wards in 16 NHS Trusts, for the baseline and early intervention phases of the trial, using a standardised reliable and sensitive tool, the Hand-Hygiene Observation Tool [1] (www.npsa.nhs.uk/cleanyourhands). Data were collected on staffing levels, numbers of hand-hygiene opportunities and whether they were high/low risk, pre-/post-contact. Analysis was by descriptive statistics and Spearman rank correlations.

**Results:** Overall HHC was much higher at 71% than commonly reported, even during the prolonged baseline phase of the trial. There was no difference between doctors and nurses (73% HHC) or high and low risk activities (68%). Although the study confirmed an inverse relationship between compliance & patient:nurse ratio (r = −0.19, p = 0.007) it showed compliance rose with the number of hand-hygiene opportunities per patient (r = 0.208, p = 0.000)

**Conclusions:** This is the largest data set of hand-hygiene observations in the literature. Analysis shows much better hand-hygiene behaviour than previously reported. Potential explanations of this are the larger and arguably more representative data set and/or the use of a more rigorously standardised measure of compliance. However, we think the difference is most likely to be due to a significant improvement in hand-hygiene behaviour in the UK, reflecting a major change in NHS culture.

**Reference(s)**


**Production of the WHO-recommended alcohol-based handrub formulations in 11 different sites worldwide**

B. Allegranzi*, S. Bagheri Nejad, H. Sax, E. Mathai, H. Richet, D. Pittet (Geneva, CH)

**Objective:** Production of the WHO-recommended alcohol-based handrub formulations (ABHF) at the point of care is an essential element of the WHO multimodal hand hygiene (HH) improvement strategy. Tools were developed by WHO to help healthcare settings to make this system change, including a guide to the local production of 2 formulations. We evaluated the feasibility, quality control, and local production costs in different sites worldwide.

**Methods:** A survey was conducted in July-September 2008 to gather data on the WHO-recommended ABHF local production in 11 sites testing the WHO strategy in Bangladesh, Costa Rica, Egypt, Hong Kong SAR, Kenya, Mali, Mongolia, Pakistan (2 sites), Saudi Arabia, and Spain. Questions related to equipment used, staff involved in production, sourcing and cost of ingredients, quality control of the final product, adequacy of facility for preparation and storage, and distribution and end use; open-ended questions on lessons learnt were also included. Quality checks by gas chromatography and the titrimetric method were performed on the final products.

**Results:** All sites completed the survey. Apart from Bangladesh and Pakistan, all sites manufactured the ABHF based on ethanol. In 7 hospitals, local production was carried out successfully at the pharmacy. In Bangladesh, Costa Rica, Hong Kong SAR and Saudi Arabia, ABHF was produced by a pharmaceutical company for distribution to several facilities across the country. Production volumes varied significantly according to local needs (10–600,000 litres/month). In a few cases, difficulties arose for the local procurement of some ingredients and dispensers. Facilities for production and storage were considered adequate in all sites but two (Mali and Pakistan); the ABHF was shown to be stable, even at tropical temperatures (up to 19 months). Quality checks on samples from 7 sites yielded optimal results. Good tolerability and acceptability by healthcare workers were reported. Cost assessment was conducted in 5 sites and ranged between US$0.30–0.50 per 100 ml.

**Conclusion:** Local production of the WHO-recommended ABHF is feasible in different settings worldwide, despite some procurement obstacles. The final product was well-tolerated and very inexpensive compared to marketed formulations. After a test phase, 5 countries have decided to scale-up to national production. Local production is a very promising approach to make ABHF available in many more settings, especially those with limited resources.

**Successful implementation of the WHO multimodal hand hygiene improvement strategy and tools: a survey of 230 hospitals worldwide**

B. Allegranzi*, S. Bagheri Nejad, H. Sax, E. Mathai, H. Richet, D. Pittet (Geneva, CH)

**Objective:** In 2006, the WHO multimodal hand hygiene (HH) improvement strategy and more than 40 implementation tools were made available to any healthcare setting (HCS) worldwide following web registration. We evaluated the use of the strategy and tools and their usefulness and importance for HH improvement and impact on HH practices.

**Methods:** In January 2008, all registered HCS (n = 329) were invited to complete an online form requesting general information with specific questions about progress with the WHO strategy and tool implementation. HCS at advanced/semi-advanced stages of implementation and having used most of the WHO tools were selected for a semi-structured interview with the project co-ordinators including both open and closed (7-point Likert scale) questions on the WHO strategy elements and tools. The objective was to receive feedback on the advantages and drawbacks of the strategy implementation, feasibility of the local production of the WHO alcohol-based handrub formulations (ABHF), and the validity and obstacles encountered in the use of the tools. Co-ordinators were also requested to send available data on key indicators such as HH compliance.

**Results:** A total of 114 responses to the web survey were received from both single hospitals and HCS networks. Among the advanced/semi-advanced sites, 47 co-ordinators were selected for interview, representing 230 hospitals from Egypt, France, Italy, Malta, Malaysia, Mongolia, Spain, and Viet Nam. The strategy was considered comprehensive, very detailed, and a successful model for other interventions by all co-ordinators; some parts were recommended for simplification. Median scores attributed to the WHO strategy elements and principal tools are shown in the table according to their ranked importance to achieve HH improvement. ABHF local production, in place in five sites, was reported to be feasible at very low cost and the product was well accepted by healthcare workers. Co-ordinators from some hospital networks (Italy,
France, Spain) reported data on HH compliance and showed an average increase of 21% after implementation.

**Conclusion:** The WHO strategy and tools were implemented autonomously and without WHO support in many HCS worldwide. As reflected by the high scores attributed by co-ordinators, their use was considered to be very helpful and essential to improve HH. Very useful feedback on local adaptation and suggestions for improvement was obtained through this evaluation.

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**Impact of monitoring hand hygiene compliance during entire care episodes for measurement of compliance rates and interpretation of comparisons**

*M. Eveillard*, H. Hitoto, F. Raymond, A. Kouatchet, L. Dubé, V. Guilletole, M.T. Prudelé, P. Brune1, A. Mercat, M.L. Joly-Guillou (Angers, FR)

**Objective:** Monitoring of hand hygiene (HH) practices and performance feedback represent one of the components which should be included in multimodal strategies implemented to improve compliance. Our objective was to assess the importance of monitoring HH compliance during series of successive contacts with patients or surroundings for measurement and interpretation of the compliance rates.

**Methods:** A direct observational study of HH compliance was performed in 4 intensive care units (ICUs) and 4 healthcare settings with non-intensive care wards (NICWs) (acute-care geriatric wards, rehabilitation units, and long-term care facilities). HH opportunities were (i) before patient contact, (ii) before aseptic task, (iii) after body fluid exposure risk, (iv) after patient contact, and (v) after contact with patient surroundings.

To assess the impact of monitoring HH compliance during entire care episodes, HH opportunities were differentiated in 2 categories: extra-series opportunities (ESO) (from the first contact to the opportunity preceding the last in the same series). Comparisons of compliance rates were performed with the chi-square test.

**Results:** In all, 1663 opportunities of HH were observed (903 in ICUs and 760 in NICWs). Among them, the proportion of ISO was 46.0% in ICUs and 22.9% in NICWs. The overall compliance was significantly higher in NICWs than in ICUs (61.2% vs. 47.5%, \( P < 10^{-6} \)). The compliance of HH was significantly higher for ESO than for ISO (67.7% vs. 28.5%, \( P < 10^{-5} \)). The compliance in ISO was significantly higher in ICUs (32.2% vs. 19.0%, \( P < 0.005 \)). If the distribution of categories of HH opportunities observed in NICWs had been the same as in ICUs, the overall HH compliance would have been 46.4% in NICWs (vs. 47.5% in ICUs). Therefore, there would have not been any difference between the 2 categories of wards.

**Conclusion:** Monitoring compliance of HH during entire care episodes in series of successive contacts is necessary to avoid a strong overestimation of the overall compliance rates. Concurrently, comparison of compliance data between wards or healthcare settings should take into account the proportion of ISO (i.e. the proportion of series of successive contacts among all contacts and the lengths of those series) included in the evaluation study.

**Comparison of hand hygiene practices between physicians and nurses**

E. Hakko*, I. Deger, S. Civil, K. Rasa, M. Cakmakci (Kocaeli, TR)

**Objective:** This observational study was aimed to determine hand hygiene practices of healthcare workers with a special interest on the comparison of compliances of physicians and nurses.

Hand hygiene practice was calculated to be significantly lower in physicians compared to nurses (\( p = 0.0001 \)).

**Comparison of hand hygiene practices between physicians and nurses**

E. Hakko*, I. Deger, S. Civil, K. Rasa, M. Cakmakci (Kocaeli, TR)

**Objective:** This observational study was aimed to determine hand hygiene practices of healthcare workers with a special interest on the comparison of compliances of physicians and nurses.

Hand hygiene practice was calculated to be significantly lower in physicians compared to nurses (\( p = 0.0001 \)).
Also, compliance to good hand hygiene practice varied at different settings. The compliance ratios before invasive procedures were 42% and 79%, after the invasive procedures were 85% and 87%; before gloving were 30% and 22%; and after taking of gloves were 77% and 87% in physicians and nurses respectively. Also compliance to good hand hygiene practice ratio while passing from one patient to another was 42% and 91% and after physical examination of the patient was 46% and 100% in physicians and nurses, respectively.

**Conclusion:** We found that the compliance to hand hygiene practices is lower in physicians when compared to nurses. This study also showed us that the weakest site of compliance is before putting on gloves which is valid for both physicians and nurses. Therefore, by this study we not only observed the present level of hand hygiene awareness and practices but also defined the sites where we could make improvements. These findings also encouraged us to perform these kinds of observational studies to tailor our future education programmes.

**P1143 Impact of teaching activities on compliance with hand disinfection of physicians and nurses**

**Y. Flammer*, H. Giger, C. Ruef (Zurich, CH)***

**Objective:** To investigate the impact of teaching interventions on the compliance with hand disinfection (HD-C) of physicians and nurses and on the consumption of alcohol based hand rub (AbHR).

**Methods:** The study was carried out on two intervention (IW) and one control ward (CW). AbHR consumption was measured weekly for six weeks. During weeks one and six, hand disinfection (HD) performance was directly observed on IW and CW. During weeks two to five, nurses and physicians attended teaching sessions on IW (nurses: 3 to 5 sessions [20 minutes each]/week, physicians: 1–2 sessions [10 minutes, general discussion of nosocomial infections, importance of HD/week]), whereas no teaching was offered to the CW. Teaching of nurses included visualising the efficacy of HD by ultraviolet light, education of 6 indications of HD, analysing daily activities for indications of HD, discussion of cases of nosocomial infections, reporting results of hand hygiene, tips on improved hand hygiene and self evaluation of hand disinfection technique by use of a UV-lamp. To analyse the effect of STS, dummy variables were created (0 and 1 representing pre-campaign and post-campaign periods, respectively).

**Results:** Compliance rates on IW are shown in the table.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Compliance with HD-C (%)</th>
<th>p</th>
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<tbody>
<tr>
<td>All indications</td>
<td>75.9°</td>
<td>0.01</td>
</tr>
<tr>
<td>Before patient contact</td>
<td>66.2</td>
<td>0.17</td>
</tr>
<tr>
<td>Before invasive procedures</td>
<td>73.7</td>
<td>0.16</td>
</tr>
<tr>
<td>After patient contact</td>
<td>92.9°</td>
<td>n.s.</td>
</tr>
<tr>
<td>After contact with body secretion</td>
<td>86.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Between patients</td>
<td>84.2°</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*p < 0.001, respectively.*

The overall HD-C of nurses was significantly improved despite an already relatively high baseline rate. HD-C on CW remained unchanged. After intervention nurses showed a significant improvement in HD after contact with body secretions and a trend towards significant improvement before patient contact and before invasive procedures. Overall compliance as well as compliance with individual indications of HD remained unchanged among physicians. While there was no significant difference between nurses and physicians regarding overall HD-C at baseline, overall compliance as well as compliance before invasive procedures and after contact with objects near patients was significantly higher among nurses than physicians. AbHR consumption was increased on IW-A from a mean of 59 ml/patient/day at baseline up to a mean of 74 ml/patient/day at baseline up to 84 ml/patient/day. AbHR consumption remained unchanged on CW.

**Conclusion:** Overall HD-C of nurses and AbHR consumption was improved by teaching, resulting in a compliance rate of >75% in all 6 indications. For selective indications HD-C of physicians was high. The teaching approach used in this study was successful in nurses but failed among physicians. Detailed analysis of the impact of teaching interventions on HD-C in individual indications and professions may help to define the goals and content of future intervention strategies.

**P1144 Short training sessions on hand hygiene, bed occupancy rates and nosocomial methicillin-resistant Staphylococcus aureus – a time-series approach at a German university medical centre**

K. Kaier*, A. Conrad, R. Bablik, U. Frank, M. Dettenskefer (Freiburg, DE)

**Objective:** Programmes to improve hand hygiene have shown to reduce nosocomial spread of and infections with methicillin-resistant S. aureus (MRSA). Furthermore, bed occupancy rates are suggested to be linked to nosocomial MRSA transmission. The aim of our study was to identify the impact of short hand hygiene training sessions (STS) and bed occupancy rates on the incidence of nosocomial MRSA.

**Methods:** A multivariate time series approach (01/2003–07/2008) using autoregressive moving average (ARMA) modelling was carried out at University Medical Center Freiburg, a 1600 bed tertiary care hospital. The monthly incidence of nosocomial MRSA cases (patients infected or colonised with MRSA that turned positive more than 48 h after admission per 1,000 patient days) was applied as the dependent variable. Bed occupancy rates of general wards and ICUs were expressed as percentage of time that beds were occupied. Data on use of alcohol-based hand rub (AbHR) was calculated in litres per 1,000 patient days. In addition, two hand hygiene campaigns focussing on STS were evaluated. The first was conducted from 09/2004 until 05/2005 (32 STS on ICUs and haematology wards). The second took place from 09/2006 until 12/2006 (17 STS on ICUs and general wards). STS included information on hand hygiene, tips on improved hand hygiene and self evaluation of hand disinfection technique by use of a UV-lamp. To analyse the effect of STS, dummy variables were created (0 and 1 representing pre-campaign and post-campaign periods, respectively).

**Results:** For the study period, a mean incidence of 0.15 nosocomial MRSA cases per 1,000 patient days was identified. The mean percentage of time that beds were occupied was 78% in general wards and 81% on ICUs. The mean use of AbHR was 55.3 litres per 1,000 patient days. Temporal increases in bed occupancy rates in general wards and ICUs were followed by increases in the incidence of MRSA (p = 0.001 and p < 0.001, respectively). Additionally, increased use of AbHR led to a decrease in MRSA incidence (p = 0.025) and both hand disinfection campaigns reduced the MRSA incidence (p = 0.038 and p < 0.001). A conclusion was that STS selectively performed in high risk areas proved to be effective in preventing nosocomial MRSA while high bed occupancy rates showed to have an effect on the incidence of nosocomial MRSA.

**P1145 e-Bug: using games to teach young children about microbes, hygiene and appropriate antibiotic use**

D. Farrell*, P. Kostokova, C. McNulty, D. Lecky (London, Gloucester, UK)***

**Background:** e-Bug is a DG SANCO funded antibiotic and hygiene teaching resource aiming to reinforce an awareness of microbes, hand and respiratory hygiene and the benefits of prudent antibiotics use among junior and senior school children across Europe. Education packs used at schools are complemented by web-based interactive games teaching the key learning outcomes of the e-Bug project. We have developed a platform game for 9–11 year old children that is fast and engaging and which uses the game mechanics to teach concepts to the player. This talk will demonstrate the game and discuss preliminary evaluation data which shows the relative success of different aspects of the design.

**Methods:** The game is broken into stages. Each stage focuses on one set of learning outcomes (for example “introduction to microbes”). Within each stage, the games rules and mechanics implement an abstract
Hand hygiene

understanding of each learning outcome. For example, the player throws soap to wash away bad microbes. Before and after each stage, the player takes part in a quiz show game. The questions are identical each time so that knowledge and attitude changes can be assessed. By doing this, we are able to identify the relative successes of each stage of the game and as a result, change any stages which are not successful.

Design Evaluation and Results: The play experience of the game has been evaluated with schools in the UK. Changes have been made to make the game easier to understand (instructions were initially given too fast) and to control (in initial versions jumping was very difficult). The knowledge and attitude change is currently being evaluated with UK schools and initial results will be presented at ECCMID.

Conclusions: Following the initial evaluation, the game will be modified as required and a fuller evaluation will be carried out in the UK, France and the Czech Republic. The final version of the game will be implemented in 9 European countries following translation.

Figure: e-Bugscreenshot.

**P1146** Association between the amount of hand antiseptics used and hospital-acquired infections in a Danish hospital

S. Laursen*, B. Kristensen, A.M. Thulstrup, J.K. Møller, B.M. Bibby (Aarhus, DK)

**Objective:** To investigate the association between the amount of hand antiseptics used and hospital acquired septicaemias

**Methods:** A time series incidence study (ecological) was carried out between January 2004 and March 2008 in all wards (n = 31) at Aarhus University Hospital, Skejby, Denmark. Different data sources were combined. Information of the monthly amount of hand antiseptics used was collected from the hospital purchasing database system, while data on bed days and time of admission were compiled from the hospital administrative data system. Data on continuous cases of bacteraemia were collected from the laboratory information system in Department of Clinical Microbiology (n = 400). A Danish version of the Centers for Disease Control's definitions of hospital acquired infections was used. The unique Danish civil registration number was used to identify patients with septicaemias. We used a vector auto regression model with the amount of hand antiseptics used and the number of septicaemias in the preceding two months (two monthly lags). The logarithm to the number of months since December 2003 was included as an exogenous variable to consider any trend over time.

**Results:** The use of hand antiseptics increased significantly from 27.3 L per 1000 bed days in the first quarter of 2004 to 81.5 L hand antiseptics per 1000 bed days in the first quarter of 2008 (p < 0.0001). There was no significant trend in the monthly number of septicaemias per 1000 bed days (p = 0.79). We found no significant association between the monthly use of hand antiseptics per 1000 bed days and the monthly cases of septicaemias per 1000 bed days (p = 0.72).

**Conclusion:** There was no association between the approximately 3-fold increase in the amount of hand antiseptics used and the incidence of septicaemias during the study period. Hand antiseptics used seem not to be an indicator for hospital acquired septicaemias.

**P1147** Surgical hand antisepsis in practice – what surgeons do and want

A. Conrad*, W. Bätz, M. Büszmann, R. Bahkir, R. Scholz, M. Detttenkofer (Freiburg, DE)

**Objective:** Surgical hand antisepsis (SHA) is important to prevent surgical site infections. In 2007 a new guideline was released in Germany (www.rki.de) recommending the use of alcoholic hand rubs for SHA which are licensed for a short application time of 60–90 seconds (EN 12791) and reduced routine hand washing with water and soap prior to SHA. The objective of this survey was to assess the practice of SHA at a tertiary care teaching hospital on the basis of existing local guidelines before revision.

**Methods:** A structured questionnaire was distributed to all departments that conduct surgeries. Additionally, observations were performed in different departments in order to assess the current practice of SHA.

**Results:** 278 questionnaires were completed, most of them by surgeons (54.7%) and operating theatre staff (37.4%). Knowledge of SHA was mainly acquired during schooling (74.1% of respondents). However, the local guidelines were only known to 56.5%, 43.2% stated to wash hands with water and soap prior to every surgical intervention, 56.1% just in case of soiling. In compliance with the valid guideline, 83.1% of the respondents stated to apply the disinfectant 3 minutes, 6.8% less and 7.9% longer than 3 minutes. 71.2% reported to rub the disinfectant until the skin has dried after disinfection, while 23.1% let the skin air-dry. 80.6% of the respondents quoted that time pressure is a major obstacle in performing proper SHA and 58.6% complained about dry skin correlated with routine SHA. A reduction of the application time (90 seconds) would be appreciated by 70.5%. In 47 observations incorrect disinfection technique was identified in 19.1%. In 12.7% of the observations, the disinfectant was not rubbed up to the elbow crease but limited to the forearms or hands. The application time was 3 minutes in 41.3%, less than 3 minutes in 13% and longer in 34.8%. A timer to control application time was used in only 21.4%; there was no timer available at all in 33.3% of the observations and none of the available timers had the ability to be adjusted for 90 seconds.

**Conclusions:** This survey identified limitations regarding the current practice of SHA. Because of time pressure the majority of theatre staff would appreciate the introduction of a shortened SHA. To guarantee high standards, the infrastructure of SHA has to be optimised e.g. suitable timers, skin friendly disinfectants with short application time (EN 12791) and staff training according to revised guidelines.

**P1148** Evaluation of in vitro bactericidal activity of Hibi Gel Hand Rub+, Hibi Liquid Hand Rub+ and Hibiscrub hand disinfectants on multidrug-resistant bacterial pathogens

C. Nonhoff*, S. El Hassouni, M. Struelens (Brussels, BE)

**Objectives:** To evaluate the in-vitro activity of a novel gel formulation of hand rub disinfectant (Hibi Gel Hand Rub+) in comparison with liquid hand rub formulation (Hibiscrub+) and antimicrobial soap hand wash formulation (Hibiscrub8) on clinical isolates of antibiotic-resistant bacteria and Candida albicans.

**Methods:** Testing of bactericidal activity was performed by using the quantitative suspension test method, according to the European Standard EN 1276. The dilution-neutralisation method was used to test Hibiscrub. A collection of clinically relevant strains was selected with a special emphasis on multidrug resistant bacterial pathogens related to recent epidemics of healthcare associated (HA) infections and included HA-methicillin-resistant *Staphylococcus aureus* (MRSA) strains (n = 5), vancomycin-resistant (VISA) MRSA strains and -intermediate (VISA) MRSA strains (n = 2), vancomycin-resistant *Enterococcus spp* strains (VRE) strains (n = 3), *Candida albicans* strains (n = 1) and extended-spectrum β-lactamase (ESBL)-producing...
Enterobacteriaceae strains (n=6). One C. albicans isolate was also tested.

Results: Hibi Gel Hand Rub+ and Hibi Liquid Hand Rub- both demonstrated a reduction factor (RF) >5 within 15 s against all test bacteria and Candida strains with no growth detected on plates after any reaction time. The Hibiscrub disinfectant soap formulation showed a slower killing activity on Gram-positive strains and Candida. Effective bactericidal activity (RF >5) of Hibiscrub was only achieved after 1 to 3 min of reaction time with some MRSA, VISA and VR-E. faecium strains and effective fungicidal activity against C. albicans was noted only after 3 min. All three products were highly bactericidal against the multidrug resistant strains of Enterobacteriaceae and non-fermenter Gram-negative rods.

Conclusion: Hibi Liquid and Gel Hand Rub+ disinfectants showed equivalent in vitro bactericidal activity. Both met the requirements of the European Standard in terms of time-dependent microbial reduction factor. Equally rapid killing was observed with clinically relevant antibiotic-resistant strains and quality control bacterial strains.

Study of effectiveness and antimicrobial activity of an alcohol-free, non-rinse antiseptic combination containing quaternary ammonium compounds (0.35%) and to show its advantages as active substances polyhexamethylene guanidine phosphate (0.8%) and triclosan (0.05%) in emergency situations.

Methods: The aim of this study was to investigate effectiveness and biocidal activity of an alcohol-free, non-rinse antiseptic combination containing as active substances polyhexamethylene guanidine phosphate (0.8%) and quaternary ammonium compounds (0.35%) and to show its advantages in respect to alcohol-based formulations for hygiene skin disinfection in emergency situations.

Objectives: After an emergency, it can be difficult to find running water. However, it is still important to clean your hands to avoid infection. The aim of this study was to investigate effectiveness and biocidal activity of an alcohol-free, non-rinse antiseptic combination containing as active substances polyhexamethylene guanidine phosphate (0.8%) and quaternary ammonium compounds (0.35%) equivalent in vitro bactericidal activity. Both met the requirements of the European Standard in terms of time-dependent microbial reduction factor. Equally rapid killing was observed with clinically relevant antibiotic-resistant strains and quality control bacterial strains.

Methods: The effectiveness of the formulation and its sustained effect were tested by the swab method in a cross-over design with 40 volunteers in 5 hospitals in St. Petersburg. Biocidal activity was tested against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Mycobacterium B5 and Hepatitis B virus using standard cambric test-objects method.

Results: The level of natural microflora decreased for 98.5 at hygienic hand treatment with 2 ml of the formulation and for 98.8 at hygienic hand treatment with 3 ml of the formulation. The time of treatment was at least 1 minute in both cases. It was also demonstrated that the combination had a 100% 3-hours long sustained effect and was bactericidal against S. aureus, E. coli, P. aeruginosa and viralicidal against Hepatitis B virus in 15 seconds, fungicidal against C. albicans and tuberculocidal against Mycobacterium B5 in 1 minute and virulicidal against Poliovirus type 1 in 3 minutes.

Conclusion: The present formulation demonstrated a wide spectrum of antimicrobial activity and excellent acceptance. Due to its unique properties, it meets all regulatory requirements for multipurpose skin sanitizers. On the contrast to the alcohol-based skin antiseptics, it is flame retardant, doesn’t have any skin irritating effect even at a frequent use and has a 3 hours-long sustained effect. It is powerful to prevent spreading of infection among people in emergency conditions when clean water is unavailable.
**Diagnosis of bacterial infections**

**Methods:** We prepared recombinant proteins, PVL and the homologues (gamma-haemolysin, PVL variant). We produced some ascites containing anti-PVL monoclonal antibodies and selected specific clones that recognized PVL without cross-reacting with the homologues, by using the recombinants. Antibody-sensitised latex was prepared by coating polystyrene beads with an optimum concentration of the specific antibody.

We collected 64 *S. aureus* clinical and laboratory strains, with or without PVL gene as confirmed by PCR method. Each strain was cultured in 3 mL of medium for 18 hours at 37°C with continuous shaking. The culture supernatant was used for the RPLA assay. In this assay, 25 uL of the culture supernatant was serially diluted with reaction buffer from 1:2 to 1:128 in 96 well V-bottom microtitre plates and 25 uL of the sensitised latex suspension was added to each well, and mixed by agitation. In order to obtain an agglutination reaction, the plate was kept overnight at room temperature under moist conditions to prevent the plate from drying out. Thereafter, the end point of agglutination was determined visually.

**Results:** Thirty-five isolates carrying PVL gene were found to produce the PVL toxin, on the other hand, all of the 29 isolates without PVL gene were negative. The minimum detection level of PVL by the RPLA assay was approximately 1 nanogram per ml.

**Conclusion:** PVL expression from *S. aureus* isolates tested by the new RPLA assay correlated extremely well to PVL gene presence. Hence this assay might be a simple and useful method for the detection of PVL toxin from *S. aureus* isolates.

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**Could the *Streptococcus pneumoniae* immunochromatographic test applied to nasopharyngeal aspirate be useful for diagnosing pneumococcal pneumonia?**

*S. Athlin*, K. Strålin (Orebro, SE)

**Objectives:** In order to facilitate early diagnosis of community-acquired pneumonia (CAP) caused by *Streptococcus pneumoniae* we evaluated the rapid immunochromatographic membrane test (Binax NOW *Streptococcus pneumoniae* kit; ICT) on nasopharyngeal aspirates (NpAs). The test is validated on urine and cerebrospinal fluid according to the manufacturer.

**Methods:** In this prospective study samples were collected from 193 adults hospitalised for CAP and 64 adults with no infection as controls. ICT was applied to NpAs, which had been collected with an electronic suction device. Blood culture, urinary antigen test and culture from representative sputum were used as reference standard to identify pneumococcal aetiology. IF *S. pneumoniae* was detected with at least one of the reference methods the patient was considered having pneumococcal pneumonia. The reference standard was negative if none of these methods turned out positive. In order to identify atypical aetiology, PCR was applied to respiratory tract samples for detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* respectively, and to urinary antigen test for detection of *Legionella pneumophila*.

**Results:** The test was positive for *S. pneumoniae* on NpAs in 86 patients (45%) with pneumonia and in three controls (4.7%). As comparison the test was positive on urine samples in 45 patients (23%) with pneumonia and in one control (1.6%). In 61 patients with pneumonia and positive reference standard the ICT was positive on NpAs in 49 cases. Furthermore, the test was positive in 37 of 132 cases with negative reference standard. Thus, the sensitivity was 80% and the specificity was 72%. If cultures on NpAs were added to the reference standard, the sensitivity was 81% (59/73) and specificity 78% (93/120). Atypical bacteria were detected in 20 patients with pneumonia, of which two patients (10%) were tested ICT positive in NpAs. In this study 98 patients were treated with penicillin G, penicillin V or amoxicillin as monotherapy. Among 41 patients with positive ICT and 57 patients with negative ICT 36 patients (88%) and 38 patients (67%) respectively were cured with no change of antibiotic regime.

**Conclusion:** The relative high sensitivity in combination with the low number of positive cases among controls and patients with atypical aetiology indicate that ICT applied to NpA could be useful for diagnosing pneumococcal pneumonia. A positive ICT result supports early treatment with penicillin among patients with CAP.

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**Rapid diagnosis by antigen detection for group B *Streptococcus* carriage in pregnant women**

*A. Raglio*, M. Arosio, A. Grigis, M. Peretto, D. Fanti, M.A. Latino, A. Goglio (Bergamo, Turin, Milan, IT)

**Objectives:** In 2002, the Centers for Disease Control and Prevention (CDC) recommended that all pregnant women be screened for carriage of group B *Streptococcus* (GBS) at between 35 and 37 weeks of gestation by pre-enrichment overnight in Todd-Hewitt broth followed by subculture on a blood agar plate. Not all women, however, perform the test during pregnancy. Rapid methods are available for screening at the time of birth. The purpose of our study was to evaluate the performance of an immunochromatographic test to detect GBS antigen in pregnant women 24 hours before standard culture method.

**Methods:** Between 1 May and 30 September 2008, 176 pregnant women were monitored, antenatally or at delivery, by placing 142 vaginal and 153 rectal swab specimens into Todd-Hewitt broth with nalidixic acid and colistin (Biomerieux). After overnight incubation, the broth was subcultured onto Chromid Agar strept B (Biomerieux) and the detection of GBS antigen was performed directly from the enrichment broth, according to the kit procedure (Bionexia GBS). The discordant results were analyzed by a real-time PCR, GeneXpert GBS assay (Cepheid).

**Results:** We examined 176 pregnant women, 63 positive and 113 negative for GBS culture. Antigen confirmed all the culture positive results. Among 113 negative cultures, 9 cases were positive by antigen. Taking as reference the culture, antigen showed 100% sensitivity, 92.1% specificity, NPV 100% and PPN 87.5%. We tested by PCR 7 samples with discordant results: 4 resulted positive and 3 negative.

**Conclusions:** The detection of GBS antigen by immunochromatographic test (Bionexia GBS) allowed to identify all the positive cases and showed a NPV of 100%. PCR results suggested that the immunochromatographic test may be more sensitive than culture and allowed to a specificity increase. The reading of antigen was always easy, only in 6 cases with the growth of 3−4 GBS colonies onto Agar ChromId strepto B, the colorimetric reaction of sample-line resulted weak and this could lead to equivocal interpretation. GBS antigen detection was rapid, reliable, easy-to-perform and able to identify all the colonised pregnant women already within 24 hours after sample collection.

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**Evaluation of three media (BD Granada®, Chrom ID® and Strepto B®) for screening of recto-vaginal colonisation by group B *Streptococcus* in pregnant women and determination of the prevalence of non-haemolytic group B *Streptococcus***

I. Mansoor*, S. Dubois, M. Bachelart, C. Leroy (Tournai, BE)

**Objectives:** The CDC recommend screening of all pregnant women between 35 and 37 week of gestation for group B streptococci (GBS) by use of selective broths and subculture on sheep blood media. In Belgium Lim broths are subcultured on Granada® (GRA) media which have the pitfall that non haemolytic group B streptococci (NHGBS) will be missed as the orange red carotenoid pigment is linked with the gene producing β haemolysis. The Chrom ID Strepto B® (CIDSB) has the advantage of detecting all GBS but needs ancillary confirmation tests. In this study the detection rates of GBS for Granada®, Chrom ID® and Strepto B® (SBA); the prevalence of NHGBS and labour intensiveness were evaluated.

**Methods:** 427 specimens (majority 95%) recto-vaginal swabs) received over a 3-month period were included. Swabs were placed in Lim broths, incubated for 16−24 h at 35°C and 10μl aliquots of broths were subcultured onto GRA, CIDSB and SBA. CIDSB was incubated in air while GRA and SBA anaerobically and plates were read after 24−48 h. In case of discordant results 10 μl of broths were inoculated onto Columbia sheep blood agars (CSBA) which were incubated in 5% CO2 for 24 h
Legionella pneumophila endocarditis: a diagnostic challenge

E. Verhoye*, A. Boel, K. Van Vaerenbergh, H. De Beenhouwer (Jalst, BE)

Background: Only seven cases of prosthetic valve endocarditis (PVE) due to Legionella pneumophila are documented in literature. Laboratory diagnosis is hampered by the fact that detection of Legionella sp. in commercial automated blood culture systems might be inadequate due to insufficient growth. Legionella serology can offer a meaningful aid to this diagnostic challenge and is categorised by the modified Duke criteria as a minor criterion.

Case report: A year post aortic valve replacement for suspected but culture negative endocarditis, a 75 year old man was transferred to our hospital. He suffered from episodes of relapsing fever in spite of several therapies with different antibiotic combinations. Transoesophageal echocardiography showed vegetations on the aortic valve. Despite prolonged incubation (14 days) multiple blood cultures remained negative. The prosthetic aortic valve was replaced and sent for culture to the microbiology laboratory. After six days of incubation, the culture of the valve showed rare, slow growing colonies of Gram negative bacilli on chocolate agar medium. Nested PCR with 16S rDNA primers followed by sequencing and BLAST analysis identified L. pneumophila sp. directly from the aortic valve tissue and from the bacterial growth. This was confirmed by typing (serotype 1) and by real time Legionella sp.-specific PCR. The Binax NOW Legionella urinary antigen assay repeatedly tested negative. No Legionella sp. was detected in tracheal aspirates by molecular diagnostics. Legionella Ab titers (IFA) were very high (>1:5000). Retrospective analysis on serum, taken five months earlier, showed the same high titer. In that period, spleen infarcts and a cerebrovascular accident were reported. After the aetiological diagnosis of Legionella endocarditis was made clarithromycin was started intravenously and initially the patient showed a good recuperation. Unfortunately, 20 days postoperatively the man died of a neurological event.

Conclusion: We present a case of blood culture negative PVE, caused by L. pneumophila serotype 1. Legionella urinary antigen assay was negative but the aetiologic diagnosis was made after elective surgery by culture and PCR on the prosthetic valve. Legionella Ab titers were strongly elevated, highlighting the importance of serologic testing in case of blood culture negative cases of endocarditis.

Legionella pneumophila: Evaluation of the Oxoid Xpect™ Legionella test kit for detection of Legionella pneumophila serogroup 1 antigen in urine

J.P. Bruin, E. Scopes, M.F. Peeters, E.P.F. Leerman, B.M.W. Diederens* (Haarlem, Tilburg, NL; Basingstoke, UK)

Objectives: We evaluated a new immunochromatographic assay (the Oxoid Xpect™ Legionella urinary antigen test) for its ability to detect Legionella pneumophila serogroup 1 antigen in urine.

Methods: The Xpect™ Legionella urinary antigen test (Thermo Fisher Scientific) was evaluated against the BinaxNOW® Legionella urinary antigen test (Inverness Medical Professional Diagnostics, Scarborough, ME) using frozen urine samples. Urine samples were collected by the laboratory for Medical Microbiology and Immunology, located at the St. Elisabeth Hospital, Tilburg and the Regional Laboratory of Public Health, Haarlem, The Netherlands between 1999 and 2005, and stored at −70°C until processing was performed. Eighty-six Legionella-positive urine samples, previously confirmed by seroconversion in an IgM and/or IgG assay (SERION classic ELISA), and/or positive culture or Legionella specific PCR on a lower respiratory tract sample were included in the study. Another 87 urine samples from patients with respiratory infections other than Legionella (mainly community-acquired pneumonia from Streptococcus pneumoniae) were also incorporated. All Legionella positive and negative urine samples were read at 15 and 60 minutes. To determine the optimum incubation time regarding performance, a subset of the Legionella positive samples (n = 54) were also read at 30 and 45 min.

Results: Sensitivity was 83% after 15 min incubation, 87% after 30 min and 89% after 45 and 60 min incubation of the Xpect™ test. Specificity was 100% after 15 and 45 min incubation but 98% after 60 min incubation due to two false positive results from the Xpect™ test. Specificity of the NOW® test was 85% after 15 min incubation and 94% after 30, 45 and 60 min incubation. Specificity was 100% after both 15 and 60 min incubation. In comparison with the BinaxNOW test a calculated agreement of 96% and 97% (after 15 and 60 minutes of incubation respectively) for the OxoXpect compared to BinaxNow was found.

Conclusion: The study provided data showing that the OxoXpect™ Test has a high degree of sensitivity and specificity, with a sensitivity that increased with incubation time. By evaluating the four different read-time points we showed that reading the Xpect™ test after 45 min incubation returned results that gave optimal performance and this has now been adopted in the manufacturers instructions for use.

Mycoplasma hominis: an incidental but significant finding by routine bacteriological culture

J.B. Gertsen*, H.C. Schonheyder (Aalborg, DK)

Objectives: M. hominis is part of the normal mucosal flora and is primarily associated with infections in the genitourinary tract. Most infections occur following delivery or genitourinary instrumentation, but are also seen in immunocompromised patients. We present 4 cases diagnosed by routine bacteriological culture during a 4-year period.

Methods: Dpt. of Clinical Microbiology, Aalborg Hospital serves a population of 0.5 mio. Aerobic bacteriologic cultures are routinely carried out on 5% horse blood agar and chocolate agar (SSI Diagnostika, DK) at 35 °C in 5% CO2. The finding of translucent, pinpoint colonies after 96-120h of incubation raises the suspicion of M. hominis; support for this diagnosis is provided by Gram stain failing to reveal a distinctive micromorphology and growth of similar colonies on subculture. In the four cases a definitive identification was obtained by PCR performed at Statens Serum Institut, Copenhagen (by courtesy to Jørgen Skov).

Results: The four patients were immunocompetent women (23–56 years of age) without significant comorbidity (Table). In all patients M. hominis were obtained in pure culture. At the time of diagnosis three patients had abscesses in the genitourinary tract or endometritis. M. hominis infection was preceded by one instance of either caesarean section, vaginal hysterectomy, or a complicated vaginal delivery. The fourth
patient was admitted at term with PROM and signs of chorioamnionitis and developed endometritis postpartum. The patients did not respond to surgical drainage of the abscesses (if present) and prolonged empirical intravenous therapy with a β-lactam antibiotic and metronidazole (median 9 days). The tentative diagnosis of M. hominis prompted a change of antibiotic therapy to either moxifloxacin or clindamycin which was followed by resolution of symptoms and normalisation of CRP (median 9 days).

Conclusion: M. hominis is a rare finding by prolonged incubation of conventional blood agar. A pathogenic role of M. hominis was supported by the lack of clinical response to surgical drainage and prolonged empirical antibiotic therapy. This experience raises the pertinent question whether M. hominis infections are overlooked particularly in obstetric and gynaecological patients subsequent to vaginal birth/caesarean section or gentourinary procedures.

Four female patients with an incidental finding of M. hominis by routine bacteriological culturing:

<table>
<thead>
<tr>
<th>Patient</th>
<th>Primary procedure</th>
<th>M. hominis isolated from</th>
<th>Empirical therapy</th>
<th>Susceptibility and therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acute caesarean section</td>
<td>Corvix Placenta</td>
<td>Ampicillin &amp; metronidazole (3 days)</td>
<td>Chloramphenicol: S Clindamycin: S Tetracycline: S</td>
</tr>
<tr>
<td>2</td>
<td>Electro caesarean section</td>
<td>Intermittent abscess</td>
<td>Cefuroxime &amp; metronidazole (5 days)</td>
<td>MIC moxifloxacin: 0.036g/mL MIC ciprofloxacin: 0.094g/mL</td>
</tr>
<tr>
<td>3</td>
<td>Vaginal delivery (complicated by urinary infection)</td>
<td>Parturial fluid</td>
<td>Ampicillin (8d) → Cefuroxime (13-6) &amp; metronidazole (21d)</td>
<td>Chloramphenicol: S Clindamycin: R Tetracycline: S</td>
</tr>
<tr>
<td>4</td>
<td>Vaginal hysterectomy (metrorrhagia)</td>
<td>Vaginal abscess</td>
<td>Ampicillin &amp; gentamicin &amp; metronidazole (10 days)</td>
<td>MIC moxifloxacin: 0.036g/mL MIC ciprofloxacin: 0.023g/mL</td>
</tr>
</tbody>
</table>

The comparation of the fresh and lyophilised horse serum fertility: the effect on Mycoplasma hominis growth

T. Pijša*, G. Đakšić, M. Šablić-Vlahovac (Belgrade, RS)

Objectives: Bacteriological diagnosis of human mycoplasma infections is complicated regarding special nutritive requirements and cultivating conditions these bacteria have. A necessary component for mycoplasma growth is the fresh horse serum, the source of cholesterol, but its important flaw is that it can be easily contaminated and has a poor stability. The aim of this study was to establish the procedure for preparing long lasting, in house made media for isolation and cultivation of M. hominis, as commercial media are not easy available to many laboratories in developing countries or countries in transition.

Methods: Each of the 32 M. hominis strains were inoculated into Hayflick broth with reconstituted lyophilised horse serum (L1) and into Hayflick broth containing fresh horse serum (F). The individual bacterial cultures were then inoculated onto Hayflick agar containing the same lyophilised horse serum and the Hayflick agar prepared with the fresh horse serum. After the incubation, the colony count was performed and the bacterial growth was evaluated using the following scale: sign 0: no growth; 1: good growth (1–10 colonies) and 2: excellent growth (more than 10 colonies). The same procedure was performed: a) 3 months later using the lyophilised horse sera stored for 6 months at ±3°C (L2) and b) 6 months later, using the lyophilised horse sera stored for 9 months at ±3°C (L3) for preparation of Hayflick broth and Hayflick agar.

Results: No bacterial growth was observed for 5 strains cultivated with L1, 5 strains cultivated with L2, 7 strains cultivated with L3 and 5 strains cultivated with F. Sign 1 was reported for 15 strains cultivated with L1, 17 strains cultivated with L2, 18 strains cultivated with L3 and 16 strains cultivated with F. Sign 3 was assigned to 12 strains cultivated with L1, 10 strains cultivated with L2, 7 strains cultivated with L3 and 11 strains cultivated with F. The comparison of bacterial growth reported for each strain cultivated with L1, L2, L3 and F revealed no statistically significant difference between them (p 0.72455281; γ-2 test).

Conclusions: Our results suggest that the fertility of in house lyophilised horse serum is comparable to the fertility of the fresh horse serum, and it remains unchanged for 9 months stored at ±3°C. Therefore, we suggest lyophilised horse sera to be used instead of fresh horse sera for the preparation of Hayflick broth and Hayflick agar in a case of unavailability of commercial media.

Biotyping of Brucella melitensis using Fourier transform infrared spectroscopy

M.A. Miguel Gómez, A. Orduna Domingo, M.P. Gutiérrez Rodriguez, E.J. Marin Gil, S. Garcia de Cruz, S. Gonzalez Cabreiro, F. Menegotto, A. Cubero Rivas, M.A. Bratos Perez* (Santa Cruz de Tenerife, Valladolid, ES)

Introduction: The characterisation of biovar of B. melitensis, the most important species of the genus Brucella that produces man’s disease, has important epidemiological implications. Spectroscopy measures the interaction between electromagnetic radiation and material. When infrared radiation comes into contact with material, the energy is transferred to the atoms, ions and molecules causing tiny vibration-rotation movements in the bonds of the compounds. The inclusion of the Fourier mathematical function and subsequent use of computers for instrument control and processing, storage and visualisation of data has improved this technique.

Objective: This study has been aimed at characterising the different biotypes of B. melitensis using Fourier transform infrared spectroscopy (FTIR)

Methods: Strains type B. melitensis biotypes 1, 2 and 3 were studied. The clinical strains of B. melitensis, six corresponding to biotype 1, one of biotype 2 and five of biotype 3 were biotyped following classical methods. After killing bacteria by formaldehyde the sediment obtained by centrifugation was washed three times with distilled water, and finally was lyophilised. The dry sediment was mixed homogeneously with KBr to obtain a pellet from which the corresponding infrared reading was taken. The equipment used was a Spectrophotometer Cygmaus 100 FT-IR Spectrometer with an MTC detector and the computer software used was Win FIRST v2.0 (Mattson Instruments Inc, USA). The spectrum was recorded between 4000 and 400 cm⁻¹. The statistical analysis was performed using the SPSS v12.0 statistical package.

Results: Using the second derivative spectral data from the B. melitensis strains and specifically with the wavenumbers of the peaks selected for their greater variability, a table was drawn up to facilitate comparison. Those with the greatest variation were found to be those situated around the following wavenumbers: 770, 860, 894, 1523, 1684 and 1743 cm⁻¹. Factorial analysis was applied to the table. When applying factorial analysis to biotypes of B. melitensis we found that two factors account for 75% of the variance and with three the figure reaches 89%. Figure 1 shows the three-dimensional distribution of the factorial scores of the B. melitensis strains.

Figure 1. Distribution of Brucella melitensis biotypes.
Conclusion: FTIR may be useful in the characterisation of biotypes of B. melitensis.

Acknowledgement: This work was partially supported by Red Temática de Investigación en Brucelosis, ref. 2003/0204.

**P1160** Performance of the BioPlex 2200 Syphilis IgM automated bead immunoassay system

T.D. Ly*, C. Marencano, M. Dautigny, A. Ebel, L. Guis (Icery-sur-Seine, FR)

Background: BioPlex 2200 Syphilis IgM (Bio-Rad) is a fully automated, recombinant proteins TpN47 and TpN17 based immunoassay utilising multiplex flow technology. In this study, the performance of this new assay was compared to that of SYPHILICHECK IgM Capture (All Diag) using native antigens of Treponema pallidum.

Methods: 745 unselected serum specimens submitted for syphilis serology were screened with conventional tests: VDRL (Venereal Disease Research Laboratory) and TPHA (Treponema pallidum haemagglutination assay) or FTA-ABS (fluorescent treponemal antibody absorption). Whenever one assay was reactive, BioPlex 2200 Syphilis IgM and SYPHILICHECK IgM Capture were performed and IgM Line Immunoblot (Virotech) was used for confirmation.

Results: The results are illustrated in Tables I–III. BioPlex 2200 Syphilis IgM had an overall agreement of 88.3% versus SYPHILICHECK IgM Capture with negative agreement of 89.4% and positive agreement of 84.7%. BioPlex 2200 Syphilis IgM had an overall agreement of 92.5% versus Immunoblot, with a negative agreement of 98.3% and positive agreement of 87.1% respectively. SYPHILICHECK IgM Capture versus Immunoblot agreement was 65.5% overall, 66.5% positive, and 50% negative. Low negative agreements were due to 52 and 75 samples that were positive and equivocal respectively by SYPHILICHECK Capture and negative by Immunoblot.

Conclusion: BioPlex 2200 Syphilis IgM, the first fully-automated Syphilis IgM assay based on the use of recombinant proteins, showed better performance in comparison to SYPHILICHECK IgM Capture using native antigens microplate ELISA assay. The specificity of BioPlex 2200 Syphilis IgM is superior to that of SYPHILICHECK IgM Capture, based on the Immunoblot results obtained for the discrepant samples. The BioPlex 2200 Syphilis IgM compared with IgM Line Immunoblot had an overall agreement of 92.5%; whereas, the SYPHILICHECK IgM Capture compared to the IgM Line Immunoblot overall agreement was only 65.5%.

**Table I. BioPlex 2200 Syphilis IgM vs SYPHILICHECK IgM Capture**

<table>
<thead>
<tr>
<th>BioPlex 2200 Syphilis IgM</th>
<th>SYPHILICHECK IgM Capture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>122</td>
</tr>
<tr>
<td>Equivocal</td>
<td>4</td>
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<tr>
<td>Negative</td>
<td>53</td>
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<tr>
<td>Total</td>
<td>179</td>
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<table>
<thead>
<tr>
<th>BioPlex 2200 Syphilis IgM</th>
<th>Immunoblot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>108</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
</tr>
</tbody>
</table>

**P1161** Comparative evaluation of IMMULITE® 2000 syphilis screen assay and bioelisa Syphilis 3.0 assay for determination of antibodies to Treponema pallidum in pregnancy samples

A. Donkers* (Rotterdam, NL)

This is a comparison study between IMMULITE 2000® Syphilis Screen versus bioelisa SYPHILIS 3.0 for the detection of total antibodies specific for Treponema pallidum. IMMULITE 2000® Syphilis Screen uses one major Treponema pallidum recombinant antigen (p17). bioelisa SYPHILIS 3.0 uses three major Treponema pallidum recombinant antigens (p15, p17 and p47). All samples were tested on recomBlot Treponema IgG/IgM for confirmation.

Objective: To evaluate the performance of IMMULITE® 2000 Syphilis Screen assay versus bioelisa SYPHILIS 3.0.

Methods: A total of 681 pregnancy specimens were tested by IMMULITE 2000® Syphilis Screen, bioelisa SYPHILIS 3.0 and recomBlot Treponema IgG/IgM. IMMULITE® 2000 Syphilis Screen is a fully automated one-step chemiluminescent immunoassay. Purified p17 Treponema pallidum recombinant antigen were used in both capture and detection phase. Patient sample and the reagent are incubated together with the coated beads for 30 minutes. bioelisa SYPHILIS 3.0 is a two-step enzyme immunoassay (EIA). Purified p15, p17 and p47 Treponema pallidum recombinant antigen were used in both capture and detection phase. The results are illustrated in Tables I–III. The relative sensitivity, specificity, and agreement for bioelisa SYPHILIS 3.0 for the detection of total antibodies using pathogen specific Treponema antigens were 92%, 99.8% and 99.6% respectively; the relative sensitivity, specificity, and agreement for IMMULITE 2000® Syphilis Screen versus bioelisa SYPHILIS 3.0 was 99%. The relative sensitivity, specificity, and agreement for the IMMULITE 2000® Syphilis Screen assay against recomBlot Treponema IgG/IgM were 100%, 99.4% and 99.4% respectively; the relative sensitivity, specificity, and agreement for bioelisa SYPHILIS 3.0 assay against recomBlot Treponema IgG/IgM were 92%, 99.8% and 99.6% respectively.

Conclusions: Although IMMULITE® 2000 Syphilis Screen uses a single p17 antigen, it is more sensitive than the bioelisa SYPHILIS 3.0 using three recombinant antigens (p15, p17 and p47). IMMULITE® 2000 Syphilis Screen shown to be a highly specific and sensitive method in syphilis screening and it can be considered as alternative to other ELISA tests.

**Table II. BioPlex 2200 Syphilis IgM vs Immunoblot**

<table>
<thead>
<tr>
<th>BioPlex 2200 Syphilis IgM</th>
<th>Immunoblot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>108</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2</td>
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<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
</tr>
</tbody>
</table>

**P1162** Development of recombinant Helicobacter pylori CagA protein fragments for antibody production and characterisation

A. Klimovich*, L. Terekhina, I. Gryazeva, M. Samoylovich, A. Suvorov, V. Klimovich (St. Petersburg, RU)

Objective: Helicobacter pylori strains expressing cytotoxic CagA protein are proved to be most virulent and oncogenic. Therefore in clinical diagnostics it is necessary not only to detect the Helicobacter infection but to discriminate CagA-positive and -negative pathogen strains as well. The immunodiagnostics seem to be a good addition or even alternative to the commonly used molecular genetics methods, but lack characterised and standardised reagents. The aim of the work was to produce recombinant fragments of CagA protein, create and characterise monoclonal antibodies (MAbs) against CagA.
**P1163** Evaluation of a monoclonal-based antigen in stool enzyme immunoassay for diagnosis of *Helicobacter pylori* infection in Spanish children

S. Agudo*, T. Alarcón, P. Urruzuno, Á. Somodevilla, M. López-Brea (Madrid, ES)

**Objectives:** *Helicobacter pylori* infection can be diagnosed by invasive and no invasive methods. Invasive tests, including culture, histology and rapid urease, is necessary to do a endoscopy to obtain biopsies of the gastric mucosa and non invasive techniques such as the urea breath test, serology or detection of H. pylori antigen in stool specimen (HpSA). In this study, we use a H. pylori stool antigen test as non-invasive diagnostic methods and we compared with diagnosis based on endoscopic biopsy-based methods (culture and urease test).

**Methods:** 50 samples of biopsies obtained from paediatric patients with gastrointestinal symptoms before initiation of any therapy against H. pylori, received at the Department of Microbiology (Hospital Universitario de La Princesa, Madrid) from January 2006 to November 2008, were cultured according to standard microbiological procedures and all colonies suggestive of H. pylori were tested by Gram-strain, oxidase and urease tests to confirm the identification. Children also donated a sample of stool. Stool specimens from these patients were examined by rapid STRIP HpSA. (Pylori-Strip, Coris, Bioconcept), what are commercially available enzyme-linked immunoassay assay based technology. The sensitivity and specificity were calculated for no invasive test used in this study.

**Results:** For these 50 children, 40 (80%) were diagnosed as positive and 10 (20%) were diagnosed negative for H. pylori infection by the gold standard methods (culture and urease). Whereas 37 (74.5%) were positive and 13 (26%) were diagnosed negative by the rapid STRIP HpSA test. The sensitivity and specificity were 92.5% and 100%, respectively.

**Conclusion:** stool antigen test had high sensitive and specific for diagnostic of H. pylori. The non invasive test could be used as a routine diagnostic tool in the microbiology laboratory for assessing clinical significance and eradication control of H. pylori, because it is more comfortable for the patients, especially for children and it is possible to obtain results rapidly without the need for sophisticated laboratory equipment.

**P1164** Comparison of 6 rapid assays for detection of *Helicobacter pylori* antigen in stool in children with recurrent abdominal pain – preliminary data

L. Blairón, H. Souayah, C. Moens, A. Dediste*, O. Vandenberg (Brussels, BE)

**Objective:** Some authors demonstrated a relationship between *H. pylori* (Hp) infection and recurrent abdominal pain (RAP) in children. The diagnosis of Hp infection can be assessed by either invasive (upper gastrointestinal endoscopy with histopathology and culture), or non invasive, but slow and expensive methods (C13-urea breath test, UBT). A few years ago, a cost-effective and rapid Hp stool antigen (HpSA) detection assay (ImmunoCard STAT, Meridian) was put on the market with a reported sensitivity and specificity up to 90%. Since a few months, other new HpSA assays are available. In this prospective study, we compare the performances of those new assays with these of ImmunoCard STAT and of a gold standard method in children with RAP

**Methods:** We included every consecutive children consulting with RAP. All patients underwent either an UBT or an endoscopy, and a stool collection for HpSA detection with ImmunoCard STAT, Rapid Hp STAR (Oxoid), Medcard Pylori (Medimar), Pylori Strip (Coris BioConcept), H. Pylori Antigen Test (Cortez Diagnostics), and H. Pylori Stool Card (Dima). All HpSA tests were performed in routine conditions.

**Results:** 25 paediatric patients (15 boys and 10 girls) with RAP and either an endoscopy or an UBT were enrolled in 9 months. The median age was 8.7 (extremes: 9.6 to 14.9; 95% CI 6.02–10.56), UBT was positive in 10/18 cases (55.6%); histopathology was suggestive of an Hp infection in 8/19 patients (42.1%). Overall, the prevalence of Hp infection was 52.0% (13/25). The performances of the different assays are shown in the table.

<table>
<thead>
<tr>
<th></th>
<th>Rapid Hp STAR</th>
<th>H. Pylori Antigen Test</th>
<th>Medcard Pylori</th>
<th>Pylori Strip</th>
<th>H. Pylori Stool Card</th>
<th>ImmunoCard STAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>76.9%</td>
<td>76.9%</td>
<td>84.6%</td>
<td>61.5%</td>
<td>76.9%</td>
<td>38.5%</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.3%</td>
<td>84.6%</td>
<td>88.5%</td>
<td>100.0%</td>
<td>84.6%</td>
<td>100.0%</td>
</tr>
<tr>
<td>PPV</td>
<td>83.3%</td>
<td>71.4%</td>
<td>78.6%</td>
<td>100.0%</td>
<td>71.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td>NPV</td>
<td>88.9%</td>
<td>88.0%</td>
<td>92.0%</td>
<td>83.9%</td>
<td>88.0%</td>
<td>76.5%</td>
</tr>
</tbody>
</table>

**Conclusion:** Although the majority of authors reported very good performances of HpSA in children, we observed, in routine conditions, a median sensitivity and specificity of 76.9% and 90.4% respectively, with a high variability between the different available kits. Furthermore, the previously only available ImmunoCard STAT showed the lower sensitivity. However, the present data are preliminary and the comparison will be continued with a larger number of patients.

**P1165** Diagnosis of *Helicobacter pylori* infection in Indonesian children: comparison of *Helicobacter pylori* stool antigen with enzyme-immunoassay and a new rapid test

D. Prasetyo*, H.J. Gerritsen, P. Mertens, V. Labrune, T. Leclipteux, E.J. Kuiper (Bandung, ID; Leiden, NL; Gembloux, BE)

**Aims:** To determine the prevalence of *Helicobacter pylori* (H. pylori) infection in symptomatic children and to compare a new developed rapid *Helicobacter pylori* Stool Antigen (HpSA) test with a conventional Enzyme-Immunoassay (EIA).
Methods: A cross sectional study was carried out among 102 high school children (12–18 years) with chronic abdominal pain (without diarrhoea or fever) living in Bandung, West Java Indonesia. All faeces samples were tested by a rapid test (Cores BioConcept, Gembloch Belgium) and by a conventional EIA (Amplified IDEIA Hp STAR, OXOID, United Kingdom). The principle of the rapid test is based on the homogeneous membrane system technology with latex microspheres and two different antibodies directed against H. pylori. If a sample contains H. pylori antigen, the complex formed of H. pylori antigen and conjugate remains bound to the monoclonal antibody adsorbed to the nitrocellulose and a red line develops. The result is visible within ten minutes. A stored collection of 32 faeces samples tested positive for H. pylori by HpSA (EIA, Amplified IDEIA Hp STAR, OXOID, United Kingdom) was retested with both assays and also included in the analysis.

Result: The overall prevalence of H. pylori infection among Indonesian children was 3% by EIA. There was an excellent correlation of rapid test results with EIA, except for 3 weak positive EIA samples that were negative by rapid test. Nevertheless, retesting by EIA of these three discrepant samples led to negative results. Of 32 stored faeces samples at the LUMC which were previously EIA positive tested, 25 were positive by EIA and 26 were positive by rapid test. Of 6 discrepant samples with the previous test results, all had low OD values at the first occasion and tested negative by repeated EIA. One sample was weak positive by EIA, also positive by rapid test but negative by repeated EIA. Including the results of the repeated test, the sensitivity, specificity, positive predictive value and negative predictive value of the rapid test were 96%, 99.3%, 92.3% and 99.1%, respectively.

Conclusion: The results demonstrate that the prevalence of H. pylori infection among symptomatic Indonesian children is very low and that new developed rapid test had an excellent performance.

**P1166** The importance of stool antigen test for the diagnosis of *Helicobacter pylori* infection and eradication follow-up


Objective: To compare the diagnostic accuracy of stool antigen test with urine antibody tests and serology, and to assess the eradication of *H. pylori* infection in adult patients with dyspepsia.

Methods: Fifty-one patients out of 110 (15 males, 36 females; mean age, 46.6±9.9 years) with dyspepsia referred to upper endoscopy and resulted with positive RUT, histopathological examination and UBT for *H. pylori* infection, were included between April 2006-July 2007. Patients were treated by clarithromycin, amoxicillin and PPI. Urine, stool and serum specimens from these patients were examined and evaluated pre-treatment, 6th-month and 6th-month after eradication therapy by URINELISA (Otsuka Pharmaceutical, Japan), Rapid Hp STAR (Oxoid) and *H. pylori* IgA/IgG ELISA (BIOHIT, Finland), respectively.

Results: 51 (100.0%) were diagnosed as *H. pylori* positive by the gold standard methods. In pre-treatment, 36 (70.6%) and 48 (94.1%) were *H. pylori* positive by URINELISA according to the kit’s cut off (OD 1) value and new cut off (OD 0.397) value defined by ROC analysis, respectively. 51 (100.0%) were diagnosed as *H. pylori* positive by the stool antigen (Rapid Hp STAR) test and 48 (94.1%) were positive by *H. pylori* IgA/IgG ELISA. In 6th month, 19 (37.3%) were diagnosed as *H. pylori* positive by UBT, 32 (62.7%) patients were successfully treated. 25 (49.1%) and 37 (72.6%) were positive by OD 1 and OD 0.397 URINELISA. The sensitivity, specificity, PPV and NPV were 52.6%, 79.0%, 53.1%, 31.3%, 40.0%, 40.6% and 65.4%, 37.3% respectively (K 0.054) (K 0.086).

Conclusion: We concluded that stool antigen test was more convenient to use for the diagnosis of *H. pylori* infection, treatment success of eradication therapy and also follow-up at 6 months. Also, we suggested that 6th-month results may indicate more reliable treatment success instead of 6 weeks’ follow-up results and may confirm the efficacy of OD 0.397 URINELISA results with the Rapid Hp STAR stool antigen test at 6th month besides pre-treatment.

**P1167** A two step algorithm, toxin immunomassay and stool culture, for the diagnosis of *C. difficile* infection where TcdA-TcdB+ variant strains are prevalent

B.M. Shin*, S.J. Yoo, J.G. Songer (Sanggye, KR; Tucson, US)

Objective: *Clostridium difficile* infection is mediated by two toxins, enterotoxin A (TcdA) and cytotoxin B (TcdB), and efficient and effective toxin identification is an important part of diagnosis. Enzyme immunomassay (EIA) to detect glutamate dehydrogenase (GDH), EIA or a cytotoxicity assay to detect TcdA and/or TcdB, and bacteriologic culture have advantages and disadvantages. TcdA-TcdB+ strains have become more prevalent worldwide, so a two-step algorithm, including toxin EIA and *C. difficile* culture, may be an effective alternative to improve the sensitivity and specificity of detection of toxigenic *C. difficile*.

Methods: Stool specimens (n = 1596) were examined for TcdA and TcdB via an enzyme-linked fluorescent immunomassay (ELFA, VIDAS CDAB, Bio-Merieux sa, Marcy-l’Etanol, France) and were also cultured for *C. difficile*. We amplified tcdA and tcdB from 419 *C. difficile* isolates and compared the results to those obtained with the two-step algorithm.

Results: The concordance rate between CDAB ELFA and *C. difficile* culture was 84.3% (1345/1596). The sensitivity and specificity of the CDAB ELFA, using culture and PCR for tcdA and tcdB as the standard, were 61.0% (205/336) and 94.7% (1193/1260), respectively. However, use of the two-step algorithm (CDAB ELFA and bacteriologic culture) increased sensitivity and specificity to 95.5% and 97.5%, respectively. Among culture positive cases, 20.5% (86/419) would have been missed if EIA for TcdA only had been used, and 20.5% (69/336) of PCR positive strains would have been missed had we not performed toxigenic culture. Conclusions: We recommended the two-step algorithm, using EIA to detect TcdA and TcdB and bacteriologic culture to detect *C. difficile*, as a practical method whereby the routine microbiology laboratory can confirm or rule out *C. difficile* infection. The method is reliable, conservative of time, and has good positive and negative predictable values, especially in areas where variant (TcdA-TcdB+) strains of *C. difficile* are prevalent.

**P1168** Comparison of two immunomassays for diagnosis of *Clostridium difficile* toxin A and toxin B


Objective: Enzyme immunomassay (ELFA) capable of detecting both toxin A and toxin B is strongly recommended for the diagnosis of *Clostridium difficile* associated disease in microbiology laboratories for detection of variant (toxin A-toxin B+) strains of *C. difficile*. Therefore, we evaluated two different EIAs for the diagnosis of *C. difficile* toxin A/B.

Methods: We performed bacteriologic culture for *C. difficile* and examined for toxin A and toxin B using enzyme linked fluorescent immunomassay (ELFA; VIDAS CDAB, Bio-Merieux sa, France) and ELISA (C.DIFFICILE TOX A/B II, TECHLAB, USA) with a total of 228 stool specimens. We performed toxin A and B genes PCRs from 117 *C. difficile* isolates and compared the results with those obtained with the two different EIAs.

Results: The concordance rate between ELFA and ELISA was 83.3% (190/228). The sensitivity and specificity of the ELFA and ELISA, using culture and PCR for toxin A and B genes as the standard, were 65.0%/72.1% and 71.8%/70.3%, respectively. Positive and negative predictive values were 78.4%/69.6% in ELFA and 71.8%/70.3% in
ELISA, respectively (P value >0.05). No differences were observed in results from ELFA and ELISA with variant strain of C. difficile.

**Conclusions:** The sensitivity of the ELISA was slightly higher than that of ELFA for toxin A and toxin B, but specificity and positive predictive value of the ELFA were rather higher than those of the ELISA although no statistical differences were observed. Bacteriologic culture and PCR assay for toxin gene are recommended for negative cases in both EIAs.

**PI169 Evaluation of two algorithms using GDH and toxin A&B enzyme immunoassays for rapid diagnosis of Clostridium difficile infection**

*J. Van Broeck*, J. Verhaegen, S. D’hollander, C. Hubert, S. Resseler, M. Delmee (Brussels, Leuven, BE)

**Introduction:** With toxigenic culture (TC) as gold standard, we evaluated the performances of two sets of GDH and toxin A&B enzyme immunoassays (EIA) for the rapid screening of Clostridium difficile infection (CDI) on stool specimens. A three-step protocol (1/GDH, 2/tox A&B if GDH positive, 3/toxigenic culture (TC) if tox A&B negative) was compared to a two-step one (1/GDH + tox A&B, 2/TC if not both positive or both negative).

**Materials and Methods:** Stools were from inpatients older than 2 years, suffering from diarrhoea. The first set included TOX A/B Quik Chek and C.Diff Quik Chek (GDH) (Techlab, Blacksburg VA USA), the second set Immunocard toxinn A&B and Immunocard C. difficile (GDH) (Meridian Cincinnati, Ohio USA).

A stool- cytotoxin assay (CTA) was performed with MRC5 cells. Cultures were performed on CCFA. In case of positive culture and negative CTA, colonies were tested for ‘in vitro’ toxin production (TC).

**Results:** A total of 295 stool specimens collected over a 9 months period in 2008 were tested. Twenty-eight samples were shown to contain toxigenic C. difficile by CTA and/or toxigenic culture (prevalence: 9.5%). The sensitivity, specificity, PPV and NPV of the GDH assays C.diff Quik Chek and Immunocard C. difficile were respectively: 89.3%, 90.3%, 49.9% and 98.8% and 71.4%, 94.8%, 58.8% and 96.9%. Those of Tox A/B Quik Chek and Immunocard A&B were respectively: 53.6%, 99.6%, 93.8% and 95.3% and 57.1%, 99.6%, 94.1% and 95.7%. Only one specimen gave a positive tox A&B with a negative GDH. A combined positive result of both GDH and toxins A&B was observed in 16 cases with both sets giving a PPV of 93.8%.

**Conclusion:** A two-step protocol where both GDH and toxins A&B are performed on all specimens offers no marked advantage over a three-step protocol. The latter allows the rapid result of more than 90% of the negative and more than 50% of the positive specimens with excellent NPV and PPV. Toxigenic culture is required for the remaining specimens.

**PI170 Evaluation of the performance of chromIDTM Vibrio, a new chromogenic medium for isolation and presumptive identification of Vibrio cholerae and Vibrio parahaemolyticus from clinical specimens**

*R. Eddabra*, J.M. Scheftel, Y. Piemont (Strasbourg, FR)

**Objective:** The aim of this study was to evaluate the performance of the chromID Vibrio agar (bioMérieux, France), a chromogenic medium for the detection of Vibrio cholerae (Vc) and Vibrio parahaemolyticus (Vp) and other Vibrio spp. in stool and swab specimens in comparison with TCBS medium (Bio-Rad, France).

**Methods:** A total of 91 samples including 30 fresh stool specimens (28 coming from Abidjan) were tested, and 61 artificially contaminated samples were inoculated with 10−3 dilutions of Vc and Vp. All samples were seeded on both media: the TCBS and the chromID Vibrio media directly and after enrichment step in alkaline peptone water (Bio-Rad). All bacterial strains that yielded a potentially significant growth were observed for colony colour and size and identified using VITEK 2 GN® cards (bioMérieux) or/and API ID 32E (bioMérieux).

**Results:** Out of 91 samples studied, 34 were positive to Vc including 14 from fresh stool specimens, 20 from artificial contaminations and 30 were positive to Vp only from artificial contaminations. The sensitivity for isolation of Vc in fresh stool specimens was identical for both media: 78.6%, 100% before and after enrichment respectively. However, positive test with chromID Vibrio concluded more rapidly to the presence of Vc. In the case of artificial contaminations, sensitivity of chromID Vibrio was more than important than TCBS after enrichment for Vc and for Vp before and after enrichment. In fresh stool specimens, the specificity of chromID Vibrio for screening Vc was significantly higher than TCBS (100%, 100% compared to 54.6%, 45.5% before and after enrichment, respectively), and remains important on both media for Vp (100% chromID Vibrio; 96% TCBS).

**Conclusion:** The overall sensitivity of chromID Vibrio and TCBS appeared similar for fresh stool and artificial contamination. However, chromID Vibrio medium was more specific for Vc in fresh stool specimens and presented some advantages in terms of rapidity to identify strains with morphologically typical colonies.

**PI171 Enhancement of motility ability of viable thermophilic Campylobacter in viscous condition**

*S.P. Vorawuthikunchan*, T. Wisessombat (Hatayi, Songkla, TH)

**Objective:** A novel apparatus has been developed for the detection of the four thermophilic Campylobacter commonly associated with human gastroenteritis including C. jejuni, C. coli, C. lari, and C. upsaliensis. It was found that the apparatus could detect more positive samples than did the conventional culture method. However, this apparatus for the isolation of Campylobacter from contaminated chicken products require enrichment for 18h. In this communication, the effects of chemotaxis on motility ability of viable Campylobacter to pass through a 0.45 μm pore size filter in viscous condition were investigated.

**Methods:** Reference strains including C. jejuni ATCC 33291, C. coli MUMT 18407, C. lari ATCC 43675, and C. upsaliensis DMST 19055 were used for the validation of the developed method. The initial numbers of artificially-inoculated viable cells per g of chicken meat was approximately 10−104. The constituents of mucin and bile (1:1) were obtained from bovine gallbladder from freshly slaughtered. Sodium citrate and constituents of mucin and bile were added into a soft-agar-coated membrane filter and incubated at both 37°C and 42°C for 24h. Drop plate method was used to determine numbers of viable Campylobacter at 6, 12, 18, and 24h.

**Results:** Campylobacter moved through the soft-agar-coated filter at both 37°C and 42°C. After 6h, constituents of mucin and bile at concentrations of 1, 5, and 10% demonstrated significant increase in numbers of viable cells (p <0.05). The numbers of the organisms at 42°C were higher than those at 37°C. Following the inoculation of Campylobacter (10² cfu/g), the numbers of cells at 42°C was 10⁵ cfu/ml and 10² cfu/ml at 37°C after 6 h inoculation. The highest concentration of the cells reached 10⁵ cfu/ml at 42°C and 10² cfu/ml at 37°C after 24h inoculation. When the inoculation was 10 cfu/g, the numbers of cells at 42°C was 10³ cfu/ml and 10 cfu/ml at 37°C after 6h inoculation. The numbers of cells at 42°C was 10⁵ cfu/ml and 10⁴ cfu/ml at 37°C after 24 h inoculation. However, there was no correlation between concentration and chemotactic effects of viable Campylobacter. In contrast, no significant differences were observed in 0.001–0.1 M of sodium citrate after 24 h incubation.

**Conclusion:** These results are useful for food manufactures. Furthermore, pathogen identification can provide important epidemiologic information that can aid in preventing further spread of the disease.

**PI172 Monitoring the evolution of brucellosis by using four common serological tests**


**Objective:** Brucellosis is an endemic disease in Greece. In chronic courses and relapses of the disease the clinical manifestations pose diagnostic difficulties. The aim of the study was the estimation of
serological findings of patients presented in hospital with complications of the disease, in a three-year period.

**Methods:** The serological records of 17 patients hospitalised from 2005–2007 with atypical symptoms of brucellosis, were reviewed. Blood cultures failed to detect *Brucella melitensis* in 15 out of 17 patients. The methods used were: Standard tube agglutination (STA) test, Rose Bengal (RB) agglutination test, Elisa IgG, IgM test, Immuno-capture-agglutination test (Brucella capta).

**Results:** The serological titers of 3 patients were not correlated with active disease (STA ≤ 80U, RB negative, Elisa IgM < 15U/ml, IgG 20–50U/ml, Brucellacapta ≤ 160). In the remaining 14 patients the serological titers of specific IgM antibodies (IgM Elisa) were negative and the specific IgG antibodies(IgG Elisa) were positive (80–210U/ml). These patients also had Brucellacapta test positive (ranged from 640 up and) the specific IgG antibodies(IgG Elisa) were positive (80–210U/ml).

**Conclusions:** The diagnosis of chronic brucellosis is mainly based on serological findings. However STA and RB screening agglutination tests don’t reach the best detection performance. Brucellacapta and Elisa IgG tests are reliable methods for the diagnosis of long evolution brucellosis. The combination of more than two methods establishes more accurate diagnosis.

**PI173** Relationship between Guillain-Barré syndrome and *Coxiella burnetii* infection


**Objectives:** Several bacterial and viral agents have been implicated in the pathogenesis of the Guillain-Barré syndrome, an acquired immunemediated disorder. In the *Coxiella burnetii* infection, focal neurological symptoms are rarely observed. Neurological symptoms of acute Q Fever consist of meningitis or meningo-encephalitis.

We review and evaluate two cases of Q Fever with neurological symptoms, diagnosed in our centre.

**Methods:** One patient, a 31-year old man, developed progressive mononeuritis multiplex involving the right arm and both legs. In the first stadium of the illness showed hyperreflexia. The other patient was a 51 year old man that presented a ataxia ophthalmoplexia, arreflexia in the legs and hyporreflexia in the arms. In both cases we asked for electrocardiograph and electromyogram thoracic radiological studies and serological determinations of virus and bacteria.

**Results:** The first patient was diagnosed of myelitis, and the second of Miller-Fisher syndrome. In both of them, serological procedures showed *Coxiella burnetii* phase II antibodies levels >1:2.048 for IgG and >1:32 for IgM. In one patient we underwent a lumbar puncture for cerebrospinal fluid analysis and we found a *Coxiella burnetii* IgG antibodies of 1:32 and high levels of proteins.

Fourteen days doxycyclin treatment (200 mg. daily) induced a rapid decreasing of antibodies levels, with a rapid recovery of the most of neurological symptoms.

**Conclusion:** *Coxiella burnetii* should therefore be added to the list of microorganisms capable of inducing the Guillain-Barré syndrome. Serological testing should be performed in cases of meningoencephalitis, lymphocytic meningitis, and peripheral neuropathy including Guillain-Barré syndrome and myelitis.

**PI174** Clinical significance of (1.3)-β-D-glucan detection in *Pneumocystis jirovecii* pneumonia


**Objectives:** To evaluate the clinical usefulness of Fungitell test (Associates of Cape Cod, Inc., Cape Cod, MA) – an immunological assay detecting (1.3)-β-D-glucan (BG) – as a diagnostic aid in *Pneumocystis jirovecii* pneumonia (PCP) diagnosis.

**Methods:** We studied prospectively the clinical application of Fungitell in PCP presumptive diagnosis, in patients not able to undergoing invasive diagnostic tests.

Patients were grouped as follows:

- presumptive PCP – as defined in 1993 revised case definition of AIDS
- fulfilling the following clinical characteristics: a) recent history of dyspnoea on exertion or non-productive cough; b) arterial PO2 <70; c) chest X-ray finding of bilateral interstitial infiltrate; d) no evidence of bacterial pneumonia. All the said clinical findings had to be in association with at least one of the following risk factors: HIV disease with <200 CD4 cells/mm; full-dose corticosteroid treatment for >8 weeks; haemotologic malignity treated with drugs affecting the cellular immune response.

- non-PCP pneumonia
- healthy volunteers.

BG assay was performed according to manufacturer’s instructions. The reportable range was 31–500 pg/ml. Cut-off value was 80 pg/ml.

**Results:** Thirty-five patients, 14 with presumptive PCP, 10 with non-PCP pneumonia and 11 healthy volunteers, were included in the study. 7 out of 14 PCP were HIV-positive, while 7 had other risk factors. All but two (both HIV-positive) had a favourable clinical course with full response to PCP therapy. 14/14 PCP patients were BG positive, 8/9 non-PCP pneumonia were BG negative, all healthy volunteers were negative. Based on these results, sensitivity and specificity were 100% and 95%, respectively.

**Conclusions:** BG assay turned out to be very useful in strengthening the clinical suspicion of PCP. We did not find any false negative result, making the BG negative predictive value equal to 100%. We had a false positive only – not confirmed in a second sample taken 48 hours apart – in a patient with risk factors for PCP but diagnosed as having a non-PCP pneumonia. Therefore, we think Fungitell may be a useful aid for diagnosing PCP in patients not able to undergoing invasive diagnosis.

**Blood cultures**

**PI175** *S. aureus* bloodstream infection and time-to-positivity

Y. Martin, J.A. Alava, M.J. Unzaga, C. Ezeleta, C. Busto, R. Cisterna* (Bilbao, ES)

**Objective:** The aim to this study is to assess if the time to positivity (TTP) in blood culture of *S. aureus* bacteraemia correlates with the source of infection and the outcome of the patient.

**Methods:** We performed a prospective, observational study involving patients who had *S. aureus* bacteraemia between November 2003 and July 2008. When multiple cultures were positive only the shortest TTP was selected for the analysis.

**Results:** A total of 461 episodes of *S. aureus* bacteraemia were reported from 347 patients with ages between 0–92 years (median age, 67 years). 329 were methicillin sensible *S. aureus* (MSSA) and 132 were methicillin resistant *S. aureus* (MRSA). The mean age of this last group was higher (P = 0.029). The source of infection was identified in 414 bacteraemias. The most frequent source of bloodstream infection was catheter related 102 (24.6%), primary bacteraemias 77 (18.6%), skin and soft tissues infection 63 (15.2%), respiratory 37 (8.9%), urinary tract 34 (8.2%), surgical wound 16 (3.9%) and 8 (1.9%) gastrointestinal, other sources of bacteraemias were 77 (18.6%). Catheter related bacteraemia was the most common source of infection in both cases MRSA (21.9%) and MSSA infection (25.7%).

The TTP was 13.18h (range from 51 minutes to 30.58 hours). The median time of MSSA (12.78h) was shorter than MRSA (15.02h) although this difference was not significant (P = 0.08). The TTP was significantly shorter from patients with endocarditis (6.62h) compared with the rest of sources the TTP was shorter but not statistically significant (P = 0.064). If the TTP is divided into two groups, early TTP (<12h), this group is associated with endocarditis (median time 5.2h), catheter related (8.94h), respiratory (8.11h) and urinary (8.0h). The overall mortality rate was 20.6%: MRSA
Evaluation of an agglutination method (Dryspot Pneumo°, Oxoid) for the direct detection of S. pneumoniae in blood cultures

S. Vazquez, S. Rey-Cao, C. Flecha, I. Wilhelm-de Cal°
(Leganes-Madrid, ES)

Objectives: Evaluate the agglutination method Dryspot Pneumo (DS) (Oxoid) in daily routine for the direct detection of Streptococcus pneumoniae in blood cultures with the aim of reducing the time for diagnosis by 24 hours.

Methods: Within the period of October 1st 2007 and the 15th of December 2008, we studied 84 positive blood cultures for which was observed microscopically Gram positive coccid in pairs or short chains. From the positive blood culture, we extracted a volume of 2 ml that was subsequently centrifuged at 2200 rpm for 10 minutes. The test was done using the supernatant, following the manufacturer’s instructions. The results were considered positives when on the sample test site we could observe agglutination whereas the site with non sensitised reagent (control) remained with no agglutination. Indeterminate result was defined as when an agglutination could be observed in both sample and control site. Culture on plated media was used as the reference method. We determined the sensitivity, the specificity, the positive predictive value (PPV) and the negative predictive value (NPV) based on the standard statistical formulas.

Results: From the 34 confirmed cultures positives for S. pneumoniae, the DS test was found to be positive in 29 cases (85.3%). The DS test was found to be negative in all of the 50 confirmed cultures with an isolate other than S. pneumoniae (15 Enterococcus sp, 3 S. pyogenes, 1 S. bocis, 1 S. agalactiae and 30 S. viridans). No indeterminate result was found. The sensitivity, specificity, PPV and NPV were 85.3%, 100%, 100% and 90.9% respectively. The false negative strains of S. pneumoniae were found to be of different serotypes.

Conclusions: In our study, the use of the Dryspot Pneumo directly from positive blood cultures with a Gram stain showing pairs or short chains Gram positive coccid reduced the TAT for a diagnosis by 24 hrs when the result of the test was positive. We consider the Dryspot Pneumo to be a valuable tool for the rapid detection of S. pneumoniae in blood cultures.

Comparison of VersaTREK 528 and BACTEC 9240 continuous monitoring blood culturing systems for the detection of clinical isolates in a seeded study

K. Doing°, M. Fogler (Bangor, US)

Objectives: Continuous monitoring blood culture systems are used in many clinical laboratories. We evaluated the VersaTREK 528 (VT) and BACTEC 9240 (BD) blood culture systems in a seeded study comparing time to detection (TTD) and media type for the recovery of Staphylococcus aureus (20 strains) Streptococcus pneumoniae (20 strains), beta streptococci (25 strains), Neisseria meningitidis (13 strains), and Haemophilus influenzae (20 strains). ATCC strains were included as controls.

Methods: BACTEC Plus, Lytic, and PediPlus media were compared to VT standard aerobic and anaerobic media (80 and 40 mL bottles). Bottles were supplemented with 100 or 500 mL of human blood and inoculated with either 1 × 10⁵ or 1 × 10⁶ cfu/mL of bacteria (21 bottles per isolate); colony counts were performed from each dilution. Bottles were incubated in appropriate instruments with the TTD and media type recorded for each isolate. Subcultures were performed from positive bottles to ensure purity and from negative bottles after 5 days of incubation.

Results: Both systems recovered all challenge isolates, with minor differences between TTD and media noted. Isolates of H. influenzae and N. meningitidis were recovered from all aerobic media, but most failed to grow in anaerobic and all lytic media. Streptococci were detected in both systems but had a shorter TTD using BD lytic media. No differences were noted in the TTD of staphylococci. As expected, a higher inoculum shortened the TTD in both systems; however, volume of blood supplementation had no apparent impact.

Conclusions: We used a controlled inoculum to approximate the microbial load in bacteraemic patients and compared the TTD using VT standard media to BD high volume blood culture media. Optimal performance of the BD system required use of three different media, while the VT showed equivalent recovery using their standard two bottle media formulations. Indeed, the VT system is uniquely approved in the U.S. to culture low blood volumes facilitating routine use of both aerobic and anaerobic media for adult and paediatric patients. In addition, differences in instrument and media costs are significant; BD high volume resin and lytic media cost as much as 45% more than standard VT media with comparable performance. The VT system has also been cleared for body fluid and platelet culturing, and the same instrument can be used to culture mycobacteria, making this the most cost effective and versatile of the two instruments.

Time to positivity in monomicrobial and polymicrobial fungal blood cultures

L. Naumiuk°, B Rybak, A. Samet (Gdansk, PL)

Objectives: The aim of the study was to assess time to positivity (TTP) in positive fungal blood cultures who grew fungi alone or mixed with bacteria.

Methods: We analysed retrospectively positive fungal blood culture bottles (BactAlert, bioMerieux) and their TTP in tertiary care hospital from the beginning of 2007 to the end of 2008. Positive blood culture bottles were routinely subcultured on Columbia blood agar, Columbia CNA, MacConkey and Sabouraud agar according to results of microscopical examination. Microbial species were identified by classical methods and Vitek2 cards (bioMerieux). For statistical analysis Statistica software (Statsoft) was used.

Results: 139 positive culture bottles for fungi (18 from catheters) were identified from 58 patients. All grew yeasts except 3 which grew Aspergillus sp. Nearly 67% of the positive bottles belonged to FAN aerobic type. 110 bottles (79%) were monomicrobial and 29 (21%) were polymicrobial with different bacteria species recovered. Eight positive polymicrobial bottles belonged to FAN anaerobic, 19 to FAN aerobic and 2 to FAN paediatric types. The mean and median TTP for mono and polymicrobial culture were 40.8 h, 31.1 h and 21.9 h, 16.9 h respectively (Test U Mann-Whitney p < 0.005). In the polymicrobial group the median TTP of cultures which grew fungi and Gram negative and positive bacteria (n = 4) was 8.2 h and cultures with fungi and Gram positive bacteria (n = 24) had the median TTP of 19.7 h. In monomicrobial group TTP was the lowest for Candida parapsilosis followed by C. krusei, C. albicans and C. glabrata taking into account the most frequent species only.

Conclusion: In our study we identified 21% of positive blood cultures bottles which grew fungi and bacteria. TTP was half as low in the polymicrobial fungal cultures compared to monomicrobial fungal cultures. It seems that bacterial overgrowth allowed faster identification of fungi in blood cultures and did not interfere with fungal growth. Our findings also stress the need of positive blood culture bottles subculture on media which can differentiate fungi in bacterial suspension.
P1179 A comparison of the yield and speed of BACTEC Plus Aerobic/F and Peds Plus/F blood culture bottles

H.L. Nielsen*, J. Prag (Aalborg, Viborg, DK)

Objectives: To examine the benefit of adding BACTEC Peds Plus/F bottle with 40 ml fluid to a standard blood culture set consisting of a Plus Aerobic/F and a Plus Anaerobic/F blood culture bottle, both with 25 ml fluid. The paediatric bottle is supplemented with animal tissue digest (0.1% W/V) and has less sodium polyacrylate-sulfonate (0.02% W/V contra 0.05 in Plus Aerobic/F) and recent studies suggest that ordinary blood cultures only occasionally detect fastidious microorganism like Helicobacter spp. The aim of the study was to compare the yield and speed of Plus Peds Plus/F with the Peds Plus/F bottle.

Methods: During a one year period from June 2007 to July 2008 all blood cultures drawn at Viborg Hospital, Denmark, were performed using a BACTEC 9240 automated blood culture system. Blood from patients with suspected septicemia were drawn inoculated in the Plus Aerobic/F, Plus Anaerobic/F and Peds Plus/F blood culture bottles and incubated for 144 hours.

Results: The department received 7500 blood culture sets consisting of all three bottles. Each bottle respectively contained a median volume of blood of 8.5 ml, 8.5 ml and 3.5 ml in the Aerobic, Anaerobic and Peds. A total of 668 bacterial isolates were recovered per set, and of these were 491 (6.5%) of clinical importance. Of these were 279 (57%) isolates found in both Aerobic and Peds, 74 (15%) from Aerobic only, and 29 (6%) from Peds only corresponding to 2.5 times more blood volume drawn in the aerobic bottle. When bottles were positive the median time to detection were 15.6 hours for the Aerobic and 14.6 for the Peds bottle (P <0.001).

Conclusion: Compared to the Plus Aerobic/F, the Peds Plus/F bottle was superior in recovering ordinary or fastidious microorganisms. However, it detected bacteria one hour before. Addition of a third Aerobic bottle with 8−10 ml blood to the standard Aerobic/Aanaerobic isms. However, it detected bacteria one hour before. Addition of a third Aerobic bottle with 8−10 ml blood to the standard Aerobic/Aanaerobic was detected in 68 patients. With pre-incubation, blood culture results contributed important to appropriate antimicrobial management.

Table 1. Comparative times to detection in Plus Aerobic/F and Peds Plus/F blood culture bottles when both bottles were positive

<table>
<thead>
<tr>
<th>Microorganism(s)</th>
<th>No. of isolates</th>
<th>Time to detection (h), median (range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plus Aerobic/F</td>
<td>Peds Plus/F</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>50</td>
<td>16.7 (9.9−11.9)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>27</td>
<td>25.7 (13.1−44.2)</td>
<td>0.000</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>26</td>
<td>15.6 (8.2−42.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>44</td>
<td>11.9 (12.6−41.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Gram-positive faculti</td>
<td>2</td>
<td>20.7 (13.1−44.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>71</td>
<td>14.1 (8.8−66.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>20</td>
<td>15.8 (3.0−66.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Other Enterobacteriaceae</td>
<td>17</td>
<td>15.6 (8.8−41)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>4</td>
<td>16.8 (11.3−24.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Other Gram-negative bacilli</td>
<td>7</td>
<td>15.3 (12.5−30.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>11</td>
<td>49 (21.9−73.7)</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Total</td>
<td>279</td>
<td>15.6 (8.8−11.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: NS, not significant (P>0.05).

P1180 The clinical impact of pre-incubation of blood cultures at 37°C

L. van der Velden*, P. Sturm (Nijmegen, NL)

Objectives: Most laboratories do not provide a 24 hour service. Therefore, blood cultures collected outside office hours are often held at room temperature until incubation at 37°C in the laboratory. The impact of pre-incubation of blood cultures at 37°C (i.e. holding blood cultures at 37°C instead of room temperature prior to incubation in the automated blood culture system in the laboratory) on turnaround time and antimicrobial management was investigated.

Methods: The study was conducted in a 950-bed tertiary-care, university teaching hospital in Nijmegen, the Netherlands. During a 6 month period, blood cultures collected at the emergency department outside laboratory office hours were held in a 37°C incubator until transportation to the laboratory. On arrival at the laboratory, Gram stain and subcultures on chocolate agar were made from the vials before they were entered into the Bactec culture system. Times of collection, incubation in the laboratory, and availability of positive Gram stain and culture results were recorded. Gram stain and culture results were reported to the clinician as soon as these were available and antimicrobial therapy was recorded. The results of the study period were compared to the data of one year earlier when all blood cultures collected at the emergency department were stored at room temperature.

Results: During the study period 82 bacteraemias were detected in 79 patients, during the same period one year earlier 70 bacteraemias were detected in 68 patients. With pre-incubation, blood cultures turned positive on the day of collection, one day, two days, and more than two days after collection in 12%, 70%, 11%, and 7%, respectively, compared to 1%, 43%, 46% and 10%, respectively, without pre-incubation. The mean turnaround time was significantly shorter with preincubation at 37°C in blood cultures that were more than 8 hours in transport (24 hours versus 40 hours). In 31% of the patients with a bacteraemia the Gram stain results from blood cultures resulted in changes in antimicrobial management.

Conclusion: Pre-incubation of blood cultures at 37°C resulted in a significant reduction of the turnaround time. Early positive blood culture results contributed importantly to appropriate antimicrobial management.
Evaluation of the new BACTEC FX instrument and a new modified BACTEC Plus Aerobic medium

P. Beaty* (Sparks, US)

Objective: Modifications to the BACTEC™ Blood culture system are being implemented to improve the system interface (the new BACTEC FX™ instrument) and consistent product performance (a modified BACTEC Aerobic Plus™). The objective of this study is to critically assess these changes to confirm that the BACTEC system meets expected product performance criteria.

Methods: A multivariate study including instruments (BACTEC 9000 and FX), media (all BACTEC Blood culture media), blood volumes, microbial detection limits (inocula ranging from 1 to 100 CFU) and organisms diversity (a diverse set of >35 species of bacteria, yeast and fungi) was performed. The experimental design was a paired study with each variable tested in triplicate. Nonparametric statistical methods were used to analyze recovery (McNemar Test) and median time to detection (TTD) differences (Wilcoxon Test) between the test and control systems.

Results: A total of >1800 test sets are compared for recovery and TTD in the BACTEC FX and BACTEC 9240 systems. A median time to detection difference of 24 minutes was observed (the BACTEC 9240 was earlier) with a 95% confidence interval of 18 to 27 minutes. Differences in median time to detection for any given organism varied from 50 to 90 minutes, however, the majority of pairs detected within 30 minutes of each other. There was no significant difference in recovery between the two instruments. A total of 473 compliant sets were used to compare the modified Aerobic Plus to the traditional Aerobic Plus medium. The median time to detection difference was 21 minutes earlier in the new formulation with an interval range of 12 to 33 minutes. Yeasts (Candida albicans, Candida glabrata and Cryptococcus neoformans) in particular detected earlier in the modified formulation (a median TTD difference of 3.5 hours (range of 2 to 11 hours). A significant difference in recovery (detection within 5 days) was observed in the new formulation with these yeast (P = 0.0001).

Conclusion: The BACTEC is historically a high sensitivity blood culture system with a demonstrated rapid time to detection. This study demonstrates that the BACTEC FX is equivalent to the BACTEC 9240 for growth and detection of microorganisms and that the modified Aerobic Plus is equivalent to the traditional formulation with the exception of a significant increase in recovery and a decrease in time to detection of yeast.

Using digital tinctorial properties to rapidly distinguish Pseudomonas aeruginosa from other Gram-negative bacteria in bacteraemic patients

V. Nilaratanakul*, U. Rirerm, T. Chatsuwan, W. Kulwichit (Bangkok, TH)

Objectives: Proper and timely administration of antimicrobials in bacteraemic patients is crucial. P. aeruginosa demands a distinct empiric antimicrobial coverage. In this pilot study, we sought to determine staining characteristics and a rapid method to aid clinicians in making an initial antimicrobial choice upon positive signals from automated blood culture bottles.

Methods: P. aeruginosa, Escherichia coli and Klebsiella pneumoniae (ATCC strains 27853, 25922., and 27736, respectively) were smeared on surface of the slide that was divided into 4 quarters, each isolate per quarter, as controls to be compared with a positive haemoculture isolate that would be smeared on the remaining quarter. A standard Gram's staining was performed. 2 microscopic pictures of each quarter were digitally taken. 5 bacilli were then randomly selected from each picture for analysis. Maximal digital colour value of each bacterium was recorded under Adobe Photoshop CS3.

Results: 30 consecutive haemocultures, 6 of P. aeruginosa, 7 of E. coli, 5 of K. pneumoniae, 1 of mixed E. coli-K. pneumoniae, 5 of Acinetobacter baumannii and 6 of other Gram negative bacteria (Burkholderia cepacia, Enterobacter cloacae, salmonella gr.B, Serratia marcescens, Stenotrophomonas maltophilia and Moraxella sp.) were subsequently identified. 1200 bacilli, 900 from 3 controlled isolates and 300 from 30 patients' positive haemocultures, were selected. The mean value of bacterium colour for P. aeruginosa from clinical isolates were significantly different from E. coli, K. pneumoniae, E. cloacae, Salmonella gr.B and S. marcescens (P < 0.0001). 30 bacilli from 3 controlled isolates in each slide were selected for logistic regression analysis to predict whether each bacillus from patient's haemoculture in the same slide was P. aeruginosa or not. Variables were width, length and colour of each bacillus. The predicted result was shown in Table 1. In P. aeruginosa group at least 3 of 10 bacilli in pictures from patient's
Bloodstream infections

**P1183** Approaching zero rates bloodstream infections in a tertiary care neonatal intensive care unit: a multifaceted approach


**Objective:** To identify the trend of healthcare associated bloodstream infections (BSIs) and central line-associated (CLA) BSIs among a cohort of neonates admitted to the neonatal intensive care unit (NICU) and to describe the intervention program aiming to reduce the rate of infections in the NICU.

**Methods:** A prospective cohort study was conducted between January 2006 and December 2007 in a level II/III NICU at King Fahad National Guard Hospital (KFNGH). All neonates admitted to the NICU were followed to identify BSIs and CLA-BSIs rates/1000 central line days, central line utilisation ratios (UR) and risk factors for BSIs. Only laboratory confirmed LA-BSIs were considered in the study and the National Healthcare Safety Network (NHSN) definition was used for that purpose. The Hand Hygiene (HH) awareness campaign and other infection control measures were studied in relation to the trend of BSIs.

**Results:** A total of 102 neonates out of 838 followed (12.2%) had BSIs, of those 40 (4.8%) fulfilled the criteria for CLA-BSI. There was a decreasing trend of CLA-BSI rate with 3 consecutive months of zero BSIs towards the end of the study. The initiation of the HH campaign coincided with the downtrend of CLA-BSI rate. Out of the many risk factors identified in univariate analysis including decreased gestational age, decreased birth weight, using multiple CL and using umbilical catheter, prolonged CL duration was the only CL-BSI independent risk factor.

**Conclusions:** We are reporting a successful case of reducing CLA-BSI in a once risky NICU associated with better enforcement of HH guidelines and other infection control measures.

**P1184** Inter-unit comparisons of intensive care unit-acquired catheter-associated bloodstream infection rates in Cyprus and Greece

E. Kritsotakis, D. Bagatzouni, M. Alexandrou, V. Zinieri, M. Roumelakis, I. Dimitriadis, A. Gikas* (Heraklion, GR; Nicosia, Limassol, Pafos, Larnaca, CY)

**Objective:** Surveillance of intensive care unit (ICU) acquired infections has become an integral part of infection control programmes in several countries, and outcome and process indicators are increasingly used in benchmarking the quality of medical care. The objective of this study was to implement a standardised protocol for the surveillance of ICU-acquired bloodstream infections and assess its usefulness in a network of hospitals in Cyprus and Greece.

**Methods:** The study was conducted in the medical-surgical ICUs of 4 public hospitals in Cyprus and 5 public hospitals in the region of Crete in Greece. All patients admitted to the ICUs during an 11-month period were actively monitored for central line-associated bloodstream infection (CL-BSI) until their discharge or death. The US National Nosocomial Infections Surveillance system’s methods were applied. For inter-unit comparisons, the central line (CL) use ratio [(number of CL days)/(number of patient-days)] utilised as a process indicator and the CL-BSI rate [(number of primary CL-BSIs)/(number of CL-days)×1000] was used as an outcome indicator.

**Results:** During the study period, a total of 3941 patients were admitted to the study ICUs, for a mean length of stay of 5.8 days. A total of 233 primary BSIs were recorded, of which 91.8% were associated with the use of CL. Overall CL-BSI rates were high in both regions: 14.6 and 18.6 cases per 1000 CL-days in Cretan and Cypriot ICUs, respectively. CL-BSI rates varied widely among participating units, ranging from 3.7 to 22.9 cases per 1000 CL-days. CL use ratios also had significant inter-unit variation, ranging from 0.12 to 0.96. A simultaneous analysis of the two indicators is shown in the Figure. Two units (CY-1 and CR-1) were identified as having both measurements high (above the mean values represented by the solid lines), suggesting that these units need to review their practices for appropriate use of CL. One unit (CY-2) had a high CL-BSI rate despite its low CL utilisation ratio, suggesting that the unit needs to review CL insertion and maintenance practices.

**Conclusions:** ICU-acquired bloodstream infections constitute a major problem in the two study regions. Simultaneous analysis of CL-BSI rates and CL-use ratios was useful for identifying initial targets for corrective interventions to improve CL management practices and reduce infection rates in the ICUs studied.

**P1185** Risk factors for catheter-related colonisation and catheter-related bloodstream infection in chronic haemodialysis patients with cuffed tunneled central venous catheters


**Objectives:** Catheter-related bacteremia is a major cause of morbidity and mortality among catheter dependent haemodialysis patients. The aim of this study was to analyse risk factors for catheter colonisation (CC) and catheter related bloodstream infection (CRBI) in chronic haemodialysis patients with cuffed long tunneled central venous catheters.

**Methods:** From July 2003 to January 2006, we conducted a prospective study to detect CC, and establish a preemptive therapy based in catheter antibiotic lock in order to prevent development of CRBI in haemodialysis patients. Risk factors for CC and CRBI in 35 patients with 45 catheters were assessed. Patient demographic and clinical characteristics and catheter characteristics were assessed for their relationship to CC and CRBI in haemodialysis patients. These characteristics were analyzed by Kaplan-Meier survival curves. Statistical differences between survival curves were determined by the log rank test. A multivariate Cox's proportional hazards model was applied testing in the model those variables that obtained p values lower than 0.1 in the log rank test to assess the independent risk factors for CC and CRBI.

**Results:** As risk factors for CC were identified: diabetes mellitus (Hazard ratio (HR)=17.55, 95% CI, 2.91–105.99, p = 0.002), obesity (HR=13.01, 95% CI, 1.99–85.22, p = 0.007), previous CRBI episodes in the same catheter (HR=17.55, 95% CI, 1.27–108.62, p = 0.030) and duration of catheter use (HR=9.23, 95% CI, 1.22–69.62, p = 0.031). S. aureus nasal carriage (HR=9.23, 95% CI, 1.22–69.62, p = 0.031), obesity (HR=4.27, 95% CI, 1.06–17.14, p = 0.040), early detection of CRBI (HR=13.01, 95% CI, 1.99–85.22, p = 0.007), previous CRBI episodes (HR=11.77, 95% CI, 1.27–108.62, p = 0.030) and duration of catheter use (HR=4.46, 95% CI, 1.16–17.18, p = 0.030). S. aureus nasal carriage (HR=9.23, 95% CI, 1.22–69.62, p = 0.031), obesity (HR=4.27, 95% CI, 1.06–17.14, p = 0.040), early detection of CRBI (HR=13.01, 95% CI, 1.99–85.22, p = 0.007), previous CRBI episodes (HR=11.77, 95% CI, 1.27–108.62, p = 0.030) and duration of catheter use (HR=4.46, 95% CI, 1.16–17.18, p = 0.030). S. aureus nasal carriage was risk factor in CRBI episodes caused by S. aureus (p<5.85, p = 0.018) and was not risk factor
in CRBI episodes caused by other organisms different than *S. aureus* ($\chi^2 = 1.150$, P = 0.284).

**Conclusions:** Diabetes mellitus was the most important risk factor for CC. *S. aureus* nasal carriage was the most important risk factor to *S. aureus* CRBI. Obesity and early diagnosis of CC were independent factors associated with high risk of CRBI. Obesity and duration of catheterisation were common factors that increased risk of CC and CRBI. Possible preventive actions can be made according to these risk factors.

**P1186 30-day mortality in methicillin-resistant Staphylococcus aureus and methicillin-sensitive Staphylococcus aureus bloodstream infection in a Leicester cohort of patients; Panton-Valentine leukocidin gene prevalence in the same cohort**

**S. Sharma**, D. Jenkins (Leicester, UK)

**Objectives:** To compare predictors for outcomes in patients with MRSA and MSSA bacteremia; determine the frequency of the Panton-Valentine leukocidin (PVL) gene in Staph aureus bloodstream infections in Leicester and ascertain any association with clinical disease.

**Methods:** Retrospective clinical data was collected from case records and computerised laboratory records from August 2005 to January 2006. All positive staphylococcal blood cultures were identified in this period but any repeat staphylococcal positive cultures on the same patient were rejected. Data were collected on: age, gender, specialty, date of positive blood culture, MRSA colonisation, source, sepsis score, co-morbidities, 30 day mortality/survival, length of hospital stay, antibiotic treatment and antibiotic susceptibility. PCR was used to detect the PVL gene in the retrieved bacterial isolates.

**Results:** There were 120 cases Staph aureus bacteremia, (58 (48%) MRSA, 62 (52%) MSSA). 30 day mortality was 31/62 (50%) in the MRSA group, 13/58 (22%) in the MSSA group: MRSA, 62 (52%) MSSA). 30 day mortality was 31/62 (50%) in the MRSA group, 13/58 (22%) in the MSSA group: MRSA, 62 (52%) MSSA). On univariate analysis identified differences in mean age ($t=4.71$, $p=0.000$, CI: 7.46–15.2), previous colonisation with MRSA ($\chi^2=9.73$, $p=0.008$), MRSA colonisation, source, sepsis score, co-morbidities, 30 day mortality/survival, length of hospital stay, antibiotic treatment and antibiotic susceptibility. PCR was used to detect the PVL gene in the retrieved bacterial isolates.

**Conclusions:** No significant correlations were found between the presence of the PVL gene and the risk of mortality. However, a trend towards a higher mortality in patients with the PVL gene was observed.

**P1187 Risk factors for methicillin resistance and factors associated with in-hospital mortality during Staphylococcus aureus bloodstream infection: an observational study**

**M. Revest, P. Tattevin**, P.Y. Donnio, F. Fily, A. Cady, C. Arvieux, Y. Le Tulzo, C. Michelet (Rennes, FR)

**Objective:** *Staphylococcus aureus* is one of the most common actiologies of bloodstream infection (BSI) and has emerged as the leading cause of infective endocarditis. Previous studies provided conflicting data regarding risk factors for methicillin-resistance and its impact on prognosis during *S. aureus* BSI.

**Methods:** The study took place in a 1950-bed tertiary referral medical centre in Rennes, Western France. All adult patients with *S. aureus* BSI diagnosed from 1/1/2006 to 12/31/2006 were included. Standardised instrument was used for clinical data extraction from medical records.

Statistical analysis was performed using SPSS 15.0 software.

**Results:** The incidence of *S. aureus* BSI was 1.5/1000 admissions, which represented 15% of all BSI. Of 122 patients diagnosed with *S. aureus* BSI over the study period, data were available for 106 (87%). Male/female ratio was 1.1/1, mean age was 65 years. Seventy-one patients (67%) had been admitted during the previous year, and 24 (23%) had received systemic antibiotics over the last 3 months. Eleven patients (10%) had definite infective endocarditis according to Duke criteria. BSI source was assumed to be an intravascular catheter for 27 patients (25%), skin or soft tissue infection for 19 patients (18%), and was unknown for 23 patients (22%). BSI was classified as nosocomial for 73 patients (69%). Criteria for septic shock and severe sepsis were present for, respectively, 27 (25%) and 25 (24%) patients. On admission, the only significant difference between methicillin-resistant (n = 18) and methicillin-susceptible (n = 88) *S. aureus* BSI was the presence of foreign device (P < 0.001). In-hospital mortality was 22% for methicillin-susceptible and 33% for methicillin-resistant *S. aureus* BSI (P = NS). On univariate analysis, Charlson index $\geq 3$ (odds ratio 3.24 [1.85–5.66]), septic shock (OR 2.38 [1.26–4.46]), and serum creatinin $>120$ micromol/L (OR 1.74 [1.12–2.71]) were associated with mortality (P < 0.05). Only Charlson index $\geq 3$ remained predictive of mortality on multivariate analysis (OR 2.07 [1.80–2.85]; P = 0.001).

**Conclusions:** Co-morbidities, as reflected by Charlson index, and infection severity, as reflected by sepsis stage, are the most potent prognosis factors during *S. aureus* BSI.

**P1188 Risk factors and outcome of carbapenem resistant Gram-negative bacteriaemia in critically ill patients**

**M. Pratikaki, E. Platsouka**, C. Routsi, C. Sotiropoulou, C. Roussos, O. Paniai (Athens, GR)

**Objective:** To analyze the risk factors involved in the development of carbapenem resistant Gram-negative bacteriaemia (GNB) and to evaluate the outcome of infected critically ill patients.

**Methods:** All patients admitted to the 25-bed multidisciplinary ICU of Evangelismos Hospital in Athens, during a 18-month period, who developed a nosocomial GNB were studied prospectively.

**Results:** Of 855 consecutively admitted ICU patients, with $>48$ h ICU stay, 84 who developed carbapenem susceptible GNB were compared to 85 patients with carbapenem resistant GNB. The lungs were the main source of infection in both groups. The main resistant pathogens were *Acinetobacter baumannii* 32 (37.6%) and *Pseudomonas aeruginosa* 31 (36.5%). Patients infected with carbapenem resistant isolates developed ventilator-associated pneumonia more often than those with susceptible 63% vs. 34.5%, respectively, P = 0.001. Also they had a longer ICU stay (30 vs. 22.5 days, median value, P = 0.035), a longer ICU stay prior to bacteraemia onset (14 vs. 11 days, median value, P = 0.026), prolonged previous use of carbapenems (10 vs. 3 days, median value, P < 0.001) and of colistin (1 vs. 0 days, median value, P < 0.001). Mortality, although similar in patients with carbapenem resistant GNB, compared to those in patients with carbapenem susceptible (48.2% vs. 45.2%) was not statistically significant. By multivariate analysis, SOFA score on GNB onset (OR, 1.44; 95% CI 1.26–1.66; P < 0.001) and the total number of intravascular devices (OR, 2.45; 95% CI 1.16–5.15; p = 0.019) independently affected the outcome. Among the 84 patients with carbapenem susceptible pathogens, SOFA score on bacteraemia day (OR, 1.39; 95% CI 1.16–1.69, P < 0.001) and serum albumin level on bacteraemia day (OR, 0.31; 95% CI 0.11–0.88, P = 0.029) were independent factors for mortality. Among the 85 patients with carbapenem resistant GNB, SOFA score on bacteraemia day (OR, 1.46; 95% CI 1.17–1.83, P = 0.001), total number of intravascular devices (OR, 4.59; 95% CI 1.38–15.27, P = 0.013) and the presence of candidaemia (OR, 7.32; 95% CI 1.12–47.99, P = 0.038) were independently associated with mortality.

**Conclusions:** In critically ill patients, long ICU stay and prolonged previous use of carbapenems and colistin predispose to carbapenem-resistant GNB. Mortality rate was higher among patients with carbapenem resistant GNB, compared to those with carbapenem susceptible, but not statistically significant.
**P1189** Nosocomial bacteraemia due to Gram-negative bacilli: risk factors for mortality


**Objectives:** To evaluate the clinical outcomes of the patients with Gram-negative bacteraemia and to identify the risk factors for mortality.

**Methods:** A prospective observational study was performed in the 1196-bed Ankara Numune Education and Research Hospital. The patients with nosocomial Gram-negative bacteraemia were included in the study from July 2006 to June 2008. Bacteraemia was considered to be nosocomial when it was diagnosed at least 48 h after hospital admission. Gram-negative bacteraemia was defined as the presence of Gram-negative bacteria in the blood, documented by at least 1 positive hemoculture. Antibiotic therapy was considered to be appropriate if the drugs used had in vitro activity against the isolated strain.

Results: Among the 253 cases (mean age, 54.5 ± 20 years old; M:F, 159:94) of Gram-negative bacteraemia included in the study, the most frequently detected microorganisms were Escherichia coli (n = 96, 37.9%), Acinetobacter spp. (n = 54, 21.3%), Pseudomonas aeruginosa (n = 41, 16.2%), Klebsiella spp. (n = 39, 15.4%), Enterobacter spp. (n = 9, 3.5%) and S. maltophilia (n = 6, 2.3%). The mean duration of hospitalisation until the Gram-negative bacteraemia was 19.1 ± 17 (range 1–82) days. Mortality rates at 14 days and at 30 days after the bacteraemia were, respectively, 28.5% and 38.4%. Univariate analysis revealed that the risk factors for mortality at day 14 included: higher age, higher LDH and CRP, coma (OR: 3.7, CI: 1.4−9.6, p=0.007) at day 14 in logistic regression model. p=0.001), coma (OR: 3.7, CI: 1.4−9.6, p=0.007) at day 14 in logistic regression model.

Conclusions: Awareness of mortality risk factors is important for the prognosis. Appropriate antibiotic treatment could be decrease deaths associated with Gram-negative bacteraemia.

**P1190** Nosocomial versus community-acquired bloodstream infections in hospitalised patients

M. Kati, M. Kompoi*, S. Drimis, O. Zarkotou, C. Kopsari, K. Diggalaki, D. Voutsinas (Athens, GR)

**Objective:** The aim of our study was to compare characteristics and outcome in medical patients with nosocomial and community-acquired bloodstream infections (BSI).

**Patients and Methods:** A retrospective cohort study of patients with BSI who were admitted in a medical department of a tertiary hospital. All patients with at least one positive blood culture during hospitalisation were included in the study. Patient records were reviewed and data were extracted, including: age, gender, comorbidities, hospital day of positive blood cultures, isolated pathogens, C-reactive protein, erythrocyte sedimentation rate, white blood cell count and maximum body temperature on days of positive blood culture. Length of stay (LOS) and hospital outcome. Data were analyzed with Student’s t-test and logistic regression setting statistical significance at p < 0.05.

**Results:** One hundred twenty-five patients (72 males and 53 females) with BSI were included in our study. According to standard criteria, 96 patients had community-acquired and 29 nosocomial BSI. Patients with nosocomial BSI were significantly older than patients with community-acquired BSI (mean age ± SD 79.2 ± 7.0 vs. 72.1 ± 15.8, respectively, p = 0.001). Patients with community acquired BSI had a marginally higher erythrocyte sedimentation rate (ESR) and a marginally higher white blood cell count (WBC) compared with patients with nosocomial BSI (ESR: 86.1 ± 30.1 vs. 72.8 ± 33.4, respectively, p = 0.049; WBC: 16.1 ± 8.3 vs. 13.0 ± 5.3 x 10^9 cells/mm³, respectively, p = 0.059). Gram-positive microorganisms predominated in nosocomial BSIs (72.4%) and Gram-negative in community-acquired BSIs (54.2%). p = 0.024. LOS differed significantly in the two groups (17.0 ± 11.2 in the nosocomial BSI group vs. 10.6 ± 10.2 days in the community-acquired BSI group, p = 0.009). Mortality adjusted for age, sex and comorbidities did not differ significantly in the two groups of patients.

**Conclusions:** In our patient sample, nosocomial BSIs were associated with older age, a more prolonged hospital stay and a higher rate of Gram-positive microorganisms, compared with community-acquired BSIs. However, mortality did not differ significantly in the two groups.

**P1191** Incidence, clinical, microbiological features and outcome of bloodstream infections in patients undergoing haemodialysis

D. Kofteridis, M. Fysaraki, A. Valachis, M. Christofaki*, I. Aristidou, C. Mattheou, E. Daphnis, G. Samonis (Heraklion, Crete, GR)

**Objectives:** To determine the incidence, risk factors, clinical features and outcome of bloodstream infections in patients undergoing haemodialysis.

**Materials and Methods:** The records of all patients who had undergone haemodialysis at the University Hospital of Heraklion, from 1999 to 2005 were retrospectively reviewed. Multivariate analysis was used to identify risk factors among patients developing dialysis-associated bacteraemia.

**Results:** One hundred forty eight bacteraemic episodes, occurring in 102 patients, were identified. Their median age was 70 years (range 20–90). There were 53 (52%) women. The bloodstream infection rate was 0.52 per 1000 patient-days. Of the 148 episodes, 34 occurred in patients with permanent fistulae (0.18/1000 patient-days); 19 in patients with grafts (0.39/1000 patient-days); 28 in patients with permanent tunnelled central catheters (0.13/1000 patient-days), and 67 in those with temporary catheter (3.18/1000 patient-days). The relative risk for bloodstream infection of patients having artero-venous graft access was 1.84 (p = 0.029), for those with permanent central venous catheter 4.85 (p < 0.001), and for those with temporary catheter 14.88 (p < 0.001). Forty one episodes (28%) were catheter related. Gram positive bacteria were responsible for 96 episodes (65%), with S. aureus (53 out of 96; 55%) being the most frequent, followed by S. epidermidis (25 out of 96; 26%). Gram-negative organisms were responsible for 36 episodes (23%), with E. coli (14 out of 36; 39%) being the most frequent. In 14 episodes (9.5%) the infection was polymicrobial. Diabetes (p = 0.005), low serum albumin (p = 0.040) and low haemoglobin (p = 0.005) were significant risk factors for bacteraemia. Eighteen patients (18%) died during hospitalisation. Multivariate logistic regression analysis has shown that septic shock (p = 0.001) and polymicrobial infection (p = 0.041) were associated with in-hospital mortality. Mortality was not associated with the type of microorganisms involved.

**Conclusion:** The risk of bloodstream infection in patients undergoing haemodialysis is related to the type of catheter and vascular access. Presence of septic shock and polymicrobial infections predispose to unfavourable outcome.

**P1192** Nosocomial bloodstream infections in neurosurgical patients. A 5-year study


**Objectives:** Nosocomial infections are a common, serious problem in neurosurgical patients. Nosocomial blood stream infections (NBSI) are associated with increased morbidity and mortality. We analyzed NBSI in neurosurgical patients in a single centre over a five-year period.

**Methods:** A retrospective study was conducted in a 24-bed neurosurgical department between 2002–2006. Medical records and postoperative courses of 3318 patients involved in 1772 neurosurgical procedures were reviewed to determine the prevalence and characteristics of NBSI, the identity of isolated organisms, and the antimicrobial drug resistance of selected pathogens. Chi-square test was used for statistical analysis.
**Results:** Overall NBSI frequency was 3% (102/3318 patients) [24/1546 (1.5%) in non-operated patients and 78/1772 (4.4%) in operated patients (p < 0.0001)]. The median age of patients was 52.4 years (range 17–85) and the median length of stay was 39.4 days (range 5–195). The primary diagnosis was head trauma in 39% of cases followed by intracerebral haemorrhage (22%) and brain tumour (17%). Neurosurgical procedures were elective in 1144 and emergency in 628 patients. Central venous catheter was present in 63 of 102 patients with NBSI (61.7%). The most frequently isolated organism was *Klebsiella pneumoniae* (KP, 32 isolates; 22.2%) followed by *Pseudomonas aeruginosa* (PA, 24 isolates; 16.9%), *Acinetobacter baumannii* (AB, 15 isolates; 10.5%), *Enterococcus faecium* (EF, 15 isolates; 10.5%) and *Staphylococcus aureus* (SA, 14 isolates; 9.8%). Resistance rates of KP and PA were 94 and 67% to ceftazidine, 56 and 71% to piperacillin/tazobactam, 17 and 63% to imipenem, 84 and 92% to gentamicin, 50 and 83% to ciprofloxacin, respectively. All AB isolates were resistant to cefazolin and ciprofloxacin, whereas 66.6% were resistant to imipenem and 53% to gentamicin. There was no vancomycin-resistant EF, but 64.2% of SA were methicillin-resistant. In patients with NBSI mortality rate was 49% (50/102), in contrast to 5.4% (175/3216) found in those without NBSI (p < 0.0001).

**Conclusion:** NBSI constitute a serious problem in neurosurgical patients, especially postoperatively, with high frequency and mortality. The predominant organisms in our institution are multidrug-resistant Gram-negative bacteria. Understanding the patterns of neurosurgical NBSI may help to optimise infection control interventions as well as antimicrobial use.

**Reference:**

von Both U, Walti R, Ruef C (Zurich, CH)

**Objectives:** A retrospective study of enterococcal bacteraemia (EB) cases was conducted to analyze potential epidemiologic risk factors and clinical outcome with special focus on immunocompromised hosts.

**Methods:** Retrospective epidemiologic data for patients developing EB during 2006 and 2007 was collected in a 950-bed university hospital. Electronic clinical records of 104 patients (mean age 55.2 years, 67 males) were reviewed. Demographic, clinical, microbiological data as well as data regarding antibiotic treatment and subsequent outcome were determined.

**Results:** Mean length of stay (LOS) was 54 days with mean LOS of 22.6 days prior to development of EB. 64 cases of polymicrobial and 40 cases of monomicrobial culture results were detected showing *E. faecalis* in 49, *E. faecium* in 43 and non-specified enterococcal species in 12 patients. Univariate analysis showed significant association of isolation of *E. faecium* with prior antibiotic treatment (p = 0.001), organ transplant (p = 0.002) and ICU stay (p = 0.044). Multivariate analysis identified prior fluoroquinolone treatment and organ transplantation as independent risk factors for development of *E. faecium* bacteraemia, while ICU stay and underlying haematologic malignancies were strongly associated (table). Patients with *E. faecium* bacteraemia showed significant association with higher mortality (p = 0.026; OR 4.73, CI 1.2–18.6) and complication rates (p = 0.004; OR 3.86, CI 1.5–9.8). Primary bacteraemia was observed in >60% of cases while in the subgroup of transplant recipients (n = 18) surgical site infections (22.2% versus 5.8%, p = 0.024, OR 4.63, CI 95% 1.1–19.4) and intraabdominal infections (22.2% versus 5.8%, p = 0.024, OR 4.63, CI 95% 1.1–19.4) were significantly more often identified as origin of EB than in non-transplant patients. Transplant patients showed *E. faecium* in 68% (vs 28% in non-transplant patients), longer hospitalisation periods and favourable outcome despite immunesuppression (cure rate 94.4% versus 76.7% in non-transplant patients, p = 0.093).

**Conclusions:** Prior antibiotic treatment in general and exposure to fluoroquinolones in particular as well as ICU stay are significant risk factors for development of *E. faecium* bacteraemia. Organ transplant recipients are more likely to develop EB due to surgical site or intraabdominal infections. Patients with *E. faecium* bacteraemia show higher mortality and complication rates compared to *E. faecalis* infections.

**Table:** Multivariate analysis of potential risk factors for development of *E. faecium* bacteraemia

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio</th>
<th>p-value</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU stay</td>
<td>2.06</td>
<td>0.057</td>
<td>0.84–4.60</td>
</tr>
<tr>
<td>Haematologic malignancy</td>
<td>4.90</td>
<td>0.062</td>
<td>0.93–23.81</td>
</tr>
<tr>
<td>Organ transplant</td>
<td>4.06</td>
<td>0.181</td>
<td>1.59–28.39</td>
</tr>
<tr>
<td>Dialysis</td>
<td>2.98</td>
<td>0.278</td>
<td>0.65–13.5</td>
</tr>
<tr>
<td>Prior fluoroquinolone treatment</td>
<td>8.13</td>
<td>0.008</td>
<td>1.75–37.77</td>
</tr>
<tr>
<td>Prior piperacillin/tazobactam treatment</td>
<td>1.30</td>
<td>0.754</td>
<td>0.31–4.76</td>
</tr>
<tr>
<td>Prior glycopeptide treatment</td>
<td>1.82</td>
<td>0.424</td>
<td>0.42–7.00</td>
</tr>
</tbody>
</table>

**Introduction:** Infections associated with intravascular devices represent 10 to 20% of all nosocomial infections and are mostly caused by microorganisms responsible for the skin flora. Antiseptic agents are used to disinfect the skin prior to catheter insertion, to reduce the risk of device colonisation by the skin microorganisms. Nevertheless, the skin flora will rebound over time and will be able to colonise on average 13% of the inserted vascular devices. Transparent IV dressings allowing continuous observation of the insertion sites and early recognition of signs of infections, and antimicrobial dressings, suppressing skin flora re-growth, are valuable elements for best practices in IV management. This study is part of the efficacy evaluation performed with a novel dressing, the 3M® Tegaderm™ CHG (Chlorhexidine Gluconate) IV Dressing, which combines both transparency and antimicrobial activity.

**Objective:** Demonstrate antimicrobial activity of Tegaderm™ CHG gel pad against bacteria commonly associated with CA-infections by an in vitro assessment of growth inhibition.

**Methods:** Cell suspensions (approximately 10⁶ cfu/mL) were prepared from overnight growth plates of 12 bacterial strains and two yeasts (Table 1). The suspensions were streaked in three directions over the surface of a Mueller-Hinton (MH) agar plate with a sterile swab to obtain uniform growth. Pre-cut 24 mm disks from Tegaderm™ CHG dressings were placed gel side down onto the agar surfaces. Duplicate samples were prepared for each microorganism. After overnight incubation at 35°C, the diameter of the zone of inhibition was measured.

**Table 1. Strains of tested microorganisms and zones of growth inhibition**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Millimetres of zone beyond dressing</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>ATCC 51625 8.7</td>
</tr>
<tr>
<td>MRSE</td>
<td>MRSE nasal isolate #492 8.6</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>MRSA ATCC 33592 7.3</td>
</tr>
<tr>
<td>MBSA/GRSA</td>
<td>MRSA nasal isolate #849 5.7</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ATCC 27853 4.5</td>
</tr>
<tr>
<td>Enteroococcus faecium</td>
<td>VRE ATCC 700221 7.1</td>
</tr>
<tr>
<td>Enteroococcus faecalis</td>
<td>VRE ATCC 51559 7.9</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>wound isolate #23 4.3</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>ATCC 13883 4.4</td>
</tr>
<tr>
<td>Enteroococcus cloacae</td>
<td>ATCC 23357 3.6</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>ATCC 10231 7.3</td>
</tr>
<tr>
<td></td>
<td>ATCC 58716 4.9</td>
</tr>
</tbody>
</table>

**Results:** Growth inhibition around the Tegaderm™ CHG gel pad samples was observed in all MH-agar plates, varying from a minimum to 3.6 and 4.4 mm for 2 strains of *Klebsiella pneumoniae* to a maximum...
of 8.6 and 8.7 mm for 2 strains of Staphylococcus epidermidis (Table 1). Antimicrobial activity was observed also against mexitilin resistant staphylococci and vancomycin resistant enterococci and Staphylococcus aureus.

Conclusion: The Tegaderm CHG gel pad showed activity against all 14 tested strains of microorganisms.

ESBLs, AmpCs & others in Enterobacteriaceae: genes, plasmids & clones – part 2

Objective: Klebsiella pneumoniae isolates represent an important reservoir of extended spectrum β-lactamases (ESBLs) both in the hospital and in the community settings. Population structure studies in this species using an MLST typing scheme remain scarce, including the relationship with the production of ESBLs. This was analyzed in a collection of ESBL-K. pneumoniae recovered in our institution (1989–2005).

Methods: 19 K. pneumoniae previously characterised (PCR, sequencing, PFGE and phylogroups) non-related isolates, expressing the most representative ESBLs (SHV-12, TEM-4, CTX-M-10 and CTX-M-15) and were selected to perform MLST analysis (Diancourt et al. JCM 2005;43:4178–82). Allele sequences and sequence types (STs) were assigned to www.pasteur.fr/recherche/genopole/PF8/mlst.

Results: Characteristics of ESBL producing K. pneumoniae isolates and MLST typing results are shown in Table 1. All isolates have different PFGE pulstypes and belonged to the phylogroup KpII. MLST showed a high diversity, with no clear association of specific sequence types (STs) and ESBLs. This was only observed in the case of TEM-4 producing isolates, most of them belonging to ST14, previously found in K. pneumoniae isolates causing mastitis in dairy cows (Paulin-Courlinet et al, J. Dairy Sci 2007; 90:3681–9). It is noteworthy the presence of ST15 in an isolate harbouring CTX-M-15, recently described as an epidemic clone in Hungary (Damjanova et al, JAC 2008;62:978–85).

Conclusions: ESBL producing K. pneumoniae isolates showed a heterogeneous population structure without a clear relationship among STs and ESBL-types. Identified STs in our collection were previously described in animals or associated with clinical epidemic clones.

<table>
<thead>
<tr>
<th>ST</th>
<th>ESBL (no. isolates)</th>
<th>Year</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST13</td>
<td>CTX-M-15</td>
<td>2005</td>
<td>Urine</td>
</tr>
<tr>
<td>ST14</td>
<td>CTX-M-10</td>
<td>1990</td>
<td>BAS</td>
</tr>
<tr>
<td></td>
<td>TEM-4</td>
<td>1997</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>SHV-12</td>
<td>2004</td>
<td>Urine</td>
</tr>
<tr>
<td></td>
<td>TEM-4 (3)</td>
<td>1995–1999</td>
<td>Sputum, BAS (2)</td>
</tr>
<tr>
<td>ST15</td>
<td>CTX-M-15</td>
<td>2002</td>
<td>Urine</td>
</tr>
<tr>
<td>ST16</td>
<td>CTX-M-15 (2)</td>
<td>2004</td>
<td>Urine</td>
</tr>
<tr>
<td>ST20</td>
<td>CTX-M-10 (2)</td>
<td>2000–2001</td>
<td>Sputum, Urine</td>
</tr>
<tr>
<td>ST23</td>
<td>CTX-M-10</td>
<td>2000</td>
<td>Urine</td>
</tr>
<tr>
<td>ST34</td>
<td>CTX-M-10</td>
<td>2003</td>
<td>Respiratory</td>
</tr>
<tr>
<td>ST37</td>
<td>SHV-12</td>
<td>2003</td>
<td>Catheter</td>
</tr>
<tr>
<td>ST39</td>
<td>TEM-4</td>
<td>2001</td>
<td>BAS</td>
</tr>
<tr>
<td></td>
<td>SHV-12</td>
<td>2003</td>
<td>Urine</td>
</tr>
<tr>
<td>ST101</td>
<td>CTX-M-10</td>
<td>2005</td>
<td>Blood</td>
</tr>
<tr>
<td>ST111</td>
<td>CTX-M-10</td>
<td>1993</td>
<td>Blood</td>
</tr>
</tbody>
</table>

P1196 Ciprofloxacin-resistant and CTX-M-15-producing Escherichia coli from extra-intestinal infections in Italy

M. Cerquetti*, M. Giuffrè, A. García-Fernández, M. Accogli, D. Fortini, I. Lazzi, A. Carattoli (Rome, IT)

Objectives: The increasing resistance to fluoroquinolone in Escherichia coli is a major problem worldwide and the association with extended spectrum β-lactamase (ESBL) is of particular concern. This study investigated the molecular basis of resistance and phylogenetic relationship among ciprofloxacin-resistant E. coli strains isolated from human extra-intestinal infections occurring in hospital and community in Rome, Italy.

Methods: 64 ciprofloxacin-resistant strains isolated from urinary or bloodstream infections were examined for several gene markers including plasmid-mediated quinolone resistance genes (qnrA, qnrB, qnrS, and aac(6’)-Ib) and β-lactamase-encoding genes (blaCTX-M, blaSHV, blaTEM and blaOXA) by PCR and sequencing. Eighteen strains were positive for at least one of these markers and they were further investigated by analysis of the mutations in the quinolone resistance-determining region (QRDR) of gyrA, gyrB and parC genes, plasmid transferability, PCR-based replicon typing, Southern blot analysis, phylogenetic grouping, PFGE and MLST.

Results: 15/64 ciprofloxacin-resistant strains (23%) were found to be ESBL producers, and all except one were positive to the blaCTX-M15 gene, the remaining isolate harboured blaSHV-12. Most of the CTX-M15-producers also carried the blaOXA (85%), the aac(6’)-Ib-cr (78.6%) and belong to the ST131 type (71%). The blaCTX-M15 gene was located on plasmids of the IncF group, but notably, in one isolate it was integrated within the chromosome. ST131 appeared strictly associated with the following amino acid substitutions in the topoisomererase genes: Ser83Leu and Asp87Asn in gyrA; Ser90Ile and Glu84Val in parC. The remaining blaCTX-M15-positive strains all carried IncF plasmids but they belonged to the ST12, ST167, ST410 and ST405, demonstrating later transfer of this gene among different E. coli types. One ST131 isolate was positive for the blaSHV-12 gene. Two of 64 strains (3.1%), ESBL-negative, possessed both qnrB1 and aac(6’)-Ib-cr genes located on an IncHI2 plasmid and belonged to ST648.

Conclusion: The epidemic E. coli clone ST131 carrying the IncF-plasmid mediated blaCTX-M15 gene is prevalent among our isolates collected from urinary and bloodstream infections occurring in both hospitals and community. This clone combines high ciprofloxacin-resistance (MIC>32 mcg/ml) and ESBL production and is of particular concern for the treatment of human extra-intestinal infections.

P1197 Replicon typing of plasmids from Klebsiella pneumoniae and Escherichia coli clinical isolates producing CTX-M-15 extended-spectrum β-lactamase

C. Canetras, F. Nunes, L. Lito, J Melo Cristino, M. Salgado, A. Duarte* (Lisbon, PT)

Objectives: The aim of this study was to investigate the relatedness of replicons involved in the emergence and spread of Klebsiella pneumoniae and Escherichia coli carrying the blaCTX-M-15 gene.

Methods: E. coli (n=30) and K. pneumoniae (n=16) representative clinical isolates producing CTX-M-15 extended-spectrum β-lactamases (ESBLs) were collected between 2001 and 2007 in a Hospital Santa Maria, in Lisbon. Plasmid replicons were determined using the PCR-based replicon typing scheme described by Carattoli et al (2005) with specific primers for 18 plasmid replicons.

Results: Among the E. coli isolates the most frequent plasmid incompatibility group was Inc F 75.3% (22/30) with FI A and FI B replicons found on two and four isolates, respectively. Several other combinations involving F replicon were found, namely A/C, I1, I2, and Y replicons. In K. pneumoniae clinical isolates were found only the IncHI1 plasmid incompatibility group (10/16, 62.5%). The remaining 6 isolates were negative for all the replicons tested.
Conclusions: In *E. coli* isolates the bla CTX-M-15 genes are carried by plasmid incompatibility group Inc F. Overall, it is important to note a high degree of variability in plasmid profile observed among 30 *E. coli* isolates, with 13 different combinations. In 16 *K. pneumoniae* CTX-M-15 ESBL carried the IncHI1 replicons and none belonged to the IncF group. Despite the critical role of plasmids in horizontal gene transfer, this study has suggested that the bla CTX-M-15 genes cannot be readily transmitted from *E. coli* to *K. pneumoniae*.

**P1198** CTX-M-15 flanked by ISEcp1 and orf477 is the most frequent ESBL type in *E. coli* and *K. pneumoniae* from Bochum, Germany

M. Kaase†, V. Peters, F. Szabados, A. Anders, T. Sakine, S. Gutermann (Bochum, DE)

Objectives: Therapeutic options in enterobacteriaceae are limited due to an increasing prevalence of extended-spectrum β-lactamases (ESBL). A worldwide increase of CTX-M β-lactamases has been described and the insertion element ISEcp1 plays a role in the dissemination of those β-lactamases. However little is known about the molecular epidemiology of ESBL in Germany.

Methods: From January 2006 to April 2007 *E. coli* (n=102) and *K. pneumoniae* (n=75) ESBL isolates were collected from two hospitals. ESBL types were determined by PCR and sequencing. PCRs for ISEcp1 and orf477 flanking CTX-M genes were performed as described before.

Results: 59.8% of all *E. coli* ESBL isolates carried CTX-M-15, 19.6% CTX-M-1, 8.8% CTX-M-14. In *K. pneumoniae* ESBL isolates CTX-M-15 was found in 49.3% and CTX-M-1 in 45.3%. The remaining isolates carried either CTX-M-3, CTX-M-2, SHV-12 or SHV-28. ISEcp1 upstream of CTX-M was found in 91.8% and 35.1% of CTX-M-15 and CTX-M-1 genes, respectively. orf477 downstream of CTX-M was found in 98% and 59.2% of CTX-M-15 and CTX-M-1 genes, respectively.

Conclusion: ESBLs of the CTX-M-1 cluster, especially CTX-M-15, were the most frequent types in our area similar as in some but not all European countries. The high prevalence of ISEcp1 upstream of CTX-M emphasizes its role in the dissemination of this resistance gene.

**P1199** Diversity of CTX-M gene environments in *Escherichia coli* and *Klebsiella pneumoniae* nosocomial strains isolated from Russia

N. Fursoca†, S. Pryamchuk, I. Abaev, N. Shishkova, E. Pecherskikh, A. Kruglov, S. Sidorenko, E. Sietsco, L. Weigel, J. Rascheed (Obolensk, Moscow, RU; Atlanta, US)

Objectives: Characterisation of the genetic environment flanking blaCTX-M gene subtypes [blaCTX-M-1 (n=50, 64%), blaCTX-M-9 (n=24, 30%), blaCTX-M-2 (n=5, 6%)] that had been identified in *Escherichia coli* (n=54) and *Klebsiella pneumoniae* (n=25) strains isolated in Russia from 2003 to 2007.

Methods: PCR mapping, PCR-RFLP, and DNA sequencing were used for detection, localisation, and identification of blaCTX-M genes and their surrounding regions. Specific primers for detection of mobile genetic elements ISEcp1, IS26, IS903, ORF513, ORF477, and mucA were previously described (Eckert C. et al., 2006).

Results: Variability found in the genetic environment surrounding blaCTX-M genes included rearrangements of ISEcp1 mobile element upstream of blaCTX-M, short specific nucleotide sequences between ISEcp1 and blaCTX-M, and variations in the downstream flanking region. ISEcp1 mobile element (intact or partially truncated) was identified upstream of blaCTX-M in nearly all bacterial isolates under study. Another mobile element, ORF513, was found in only one strain (Fig. 1). Intact ISEcp1 was found in 39 strains; deletion of tnpA or other modifications in the 5′′ flank region in 17 strains; insertions of other IS elements into ISEcp1 (IS26, IS10, IS1, or resolvase Tn3) in 19 strains. Short nucleotide sequence insertions between ISEcp1 and blaCTX-M were found to be, generally, subtype-specific: 127 bp – for blaCTX-M-1 in *K. pneumoniae*; 48 bp – for blaCTX-M-1 in *E. coli*; 42 bp – for blaCTX-M-9; and 19 bp – for blaCTX-M-2. Three *E. coli* strains, however, were exceptions in that they contained 127 bp (n=2), and 45 bp (n=1) insertions. IS903 (intact or partially truncated) in the downstream region flanking blaCTX-M-9 was found in all bacterial isolates containing this subtype. ORF477 and mucA sequences were detected downstream of blaCTX-M-1 genes in nine isolates (Fig. 1).

Conclusion: The genetic environment of blaCTX-M genes was found to differ among CTX-M subgroups and bacterial genera suggesting differences in the mechanism of gene transmission. The presence of various mobile elements, such as IS elements, in the regions surrounding blaCTX M genes is likely key in evolution mechanisms of antibiotic resistance.

Acknowledgements: This study was done within the framework of the ISTS#2913/BTEP#62 Project.

**P1200** Characterisation of IncHI2 plasmids carrying extended-spectrum β-lactamase genes

A. Garcia-Fernández†, A. Carattoli (Rome, IT)

Objectives: IncHI2 plasmids promoted the successful spread of extended-spectrum β-lactamases (ESBL) such as CTX-M-9 and CTX-M-2 among Enterobacteriaceae of animal and human origin. Two major IncHI2 scaffolds have been identified: R478 (BX664015) originated from a clinical isolate of *Serratia marcescens* but largely prevailing in *Escherichia coli* in Europe and pAPEC-O1-R (DQS17526), prevalent in avian pathogenic and commensal *E. coli* strains in the USA, but also detected in *Salmonella* in Europe. Both R478 and pAPEC-O1-R did not carry ESBL genes. This study describes a characterisation of IncHI2 backbone among epidemic plasmids prevailing worldwide and carrying ESBL and plasmid-encoded quinolone resistance (PMQR) genes.

Methods: Twenty-one IncHI2 plasmids from *Escherichia coli*, *Enterobacter* spp, *Citrobacter freundii* and *Salmonella* spp. of human and animal origin, from 8 different countries, were analysed and
Dispersal and persistence of TEM-4 in Spain: a plasmid-clone paradigm

A. Novais*, R. Cantón, A. Valverde, F. Baquero, T.M. Coque (Madrid, ES)

Objectives: Opposite to other TEM-extended-spectrum β-lactamases, TEM-4 has a local distribution, being mainly recovered in Spain. The aim of this study was to characterise at molecular level plasmids and genetic elements involved in its spread and persistence.

Methods: Fifty TEM-4-producing Enterobacteriaceae isolates [32 K. pneumoniae (KP), 17 E. coli (EC) and 1 C. freundii (CF)] from fifty patients (1989–2004) were studied. Clonal analysis included PFGE-patterns and EC phylogroups. Antibiotic susceptibility patterns and conjugal transfer were studied. Location of blaTEM-4 was searched by hybridisation of genomic DNA (J-Ceil) with blaTEM-4/16S rDNA probes. Plasmid content was determined by using S1 nuclease (Barton’s method) and its characterisation included Inc group identification (PCR, sequencing and conjugation assays). Screening for colicins (cb, cma y cvc). The presence of class 1 integrons, Tn402 (orf5, IS1326, IS1335, IS6100) and mer-transposon derivative sequences (tnp, merA) in representative isolates was investigated by PCR. The linkage of blaTEM-4 with Tn3 sequences was also assessed by PCR.

Results: Seventeen PFGE-types (9 EC, 7 KP and 1 CF) were identified. EC isolates belonged to phylogroups A (n=3), D (n=4) and B2 (n=2). Conjugative transfer was successful in 94% of the cases, with 75% of recovered isolates belonging to phylogroups A (n=3), D (n=4) and B2 (n=2). Concurrently, the presence of colicins (cb, cma y cvc) was detected in 17% of the isolates. The occurrence of class 1 integrons, Tn402 (orf5, IS1326, IS1335, IS6100) and mer-transposon derivative sequences (tnp, merA) was investigated by PCR. The linkage of blaTEM-4 with Tn3 sequences was also assessed by PCR.

Conclusion: The spread of blaTEM-4 in our geographic area seems to be due to both epidemic clones and highly related plasmids, containing genetic platforms able to facilitate either bla dispersion (Tn3) or plasmid persistence (Tn402 and mercurial Tn derivatives) by different recombinatorial events.


R. Paniagua*, A. Valverde, F. Baquero, T.M. Coque, R. Cantón (Madrid, ES)

Objective: To further analyze extended spectrum β-lactamases (ESBLs) producing Enterobacter cloacae (ENC) and Enterobacter aerogenes (ENA) isolates recovered in our institution from 2001 to 2007 and to compare current situation with that previously found (1989–2000, Cantón et al. JCM 2002;40:1237–43).

Methods: All Enterobacter (EN) isolates recovered during the studied period were screened for ESBLs. One ESBL-isolate per patient were selected for clonal typing (XbaI-PFGE), ESBL characterisation (PCR, sequencing) and conjugal assays.

Results: A total of 54 of 2792 EN (1.9%) isolates were ESBL producers: 18 ENC (0.8%) and 36 ENA (6.4%). These figures were higher (p<0.01) than those previously found. Isolates were recovered from 50 patients (30% medical wards, 28% ICU, 18% surgical wards, and 24% outpatients). Differences between both species were found: i) a high proportion (43.8%) of ENC were obtained from outpatients, whereas 85.3% of ENA isolates had a nosocomial origin; ii) a polyclonal structure was observed in ENC (16 clones/16 isolates) whereas different clusters grouped ENA isolates (9 clones/34 isolates) with three major related clones, including the TEM-24 European epidemic clone; iii) in ENC, an increasing complexity of ESBLs (SHV-12, CTX-M-9 group, CTX-M-10 and CTX-M-15) was identified whereas ENA harboured TEM-24 and only in one case a TEM-4. Transfer of ESBL-coding plasmids was achieved in 56.2% of studied cases (9/16); iv) co-resistance was higher than in the previous period. Seventy-five percent of ENC isolates were co-resistant to sulphonamide; 68.8% to tetracycline, trimethoprim and nalidixic acid; 62.5% to ciprofloxacin; and 87.5% to one or more aminoglycosides. All ENA clones (n=9) were resistant to kanamycin, 88.9% to sulfonamide, tetracycline, trimethoprim, nalidixic acid, ciprofloxacin and tobramycin, and 77.8% to amikacin.

Conclusions: Prevalence of ESBL-EN was lower than that in Escherichia coli or Klebsiella pneumoniae. Unlike previous situation, EN isolates were polyclonal with a diverse enzyme production and mainly associated with the community. Similar to that previously reported, ENA, essentially the TEM-24 epidemic clone, were recovered in the nosocomial setting. High co-resistance rates to aminoglycosides and fluoroquinolones could facilitate the maintenance and dissemination of ESBL producing EN isolates and the corresponding resistance genes.

P1203 First identification in Italy of contemporary presence of qnrS9 element and blaTEM-116 ESBL-gene in Citrobacter freundii environmental strain

C. Forcella, M. Perrilli, C. Pellegrini, M.M. Tazio-Perez, S. Rainaldi, P. Bellio, C. Di Lisio, B. Segatore, G. Amicosante, G. Celenza* (L’Aquila, IT; GranCanaria, ES)

Objectives: Bacteria resistant to antibiotics and disinfectants have been detected in environmental compartments such as waste water, surface water, ground water, sediments and soils; resistant bacteria may be released directly into waste water systems from hospitalised and non-hospitalised patients. The aim of this study was to monitor the antibiotic resistance in the municipal sewage of a small city, in an Italian region.

Methods: 0.1 mL of the samples, collected during the period January-May 2008 from waste water of a urban sewage plant of the L’Aquila city (Italy), were diluted in sterile saline solution and plated on Nutrient Agar or MacConkey plates supplemented with nalidixic acid (6 mg/L) in order to select for potential resistance to quinolones. Genomic DNA was extracted from the strain according to the standard procedure. The blaTEM-like and qnr gene were sequenced on both strands according to the dideoxy-chain termination method by using an ABI-PRISM 310 (Applied Biosystem, Monza, Italy) automatic sequencer. MICs

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were determined by the conventional macroludition broth procedure, according to the CLSI guidelines.

**Results:** Citrobacter freundii AQ/1, selected for resistance to nalidixic acid, showed a large plasmid named pCH1333 sized more than 100 Kb and carrying both qnrB9 and blaTEM-116 gene. The plasmid was inserted by transformation into E. coli HB101 and resistance to nalidixic acid and ceftazidime was co-transferred.

MIC values were evaluated for C. freundii AQ/1 in comparison with E. coli HB101(pCH1333) and E. coli recipient cells. Both C. freundii AQ/1 and E. coli HB101(pCH1333) were resistant to piperacillin, cephalosporins (i.e. ceftazidime, MIC > 64 mg/L; cefotaxime MIC > 128 mg/L) and aztreonam (MIC > 64 mg/L). Nalidixic acid (MIC > 64 mg/L). Clavulanic acid is unable to restore the susceptibility of amoxicillin while tazobactam lowered the MIC value of piperacillin from 256 mg/L to 8 mg/L. Concerning levofloxacin and ciprofloxacin molecules, we observed the MIC value for C. freundii AQ/1 and E. coli HB101(pCH1333) of 0.25 and 0.5 mg/L, respectively.

**Conclusion:** Our findings suggest how qnr elements and blaESBL genes are easily co-transferred among Enterobacteriaceae in different environments.

**P1204 First report of TEM-52 β-lactamase in Proteus mirabilis strains from Croatia**

S. Sandelic, B. Bedenic*, D. Sijak, C. Colimon, S. Kalenic (Split, Zagreb, HR; Villeurbanne, FR)

**Objectives:** Recently an increased frequency of extended-spectrum β-lactamase (ESBL) positive P. mirabilis isolates was observed in University Hospital Split. The aim of this study was the molecular characterisation of ESBLs in P. mirabilis strains in University Hospital Split.

**Material and Methods:** Seven P. mirabilis strains with reduced susceptibility to ceftazidime were isolated from different clinical samples (mostly wound swabs) during 2007 and 2008 in University Hospital Split. ESBLs were detected by double disk synergy test. Minimum inhibitory concentrations (MICs) of wide range of antibiotics were determined by a twofold microdilution technique according to CLSI. Conjugation experiments were set up employing E. coli A15 R- strain free of plasmids and resistant to rifampin. The presence of blaESBL genes was determined by polymerase chain reaction (PCR) with primers specific for TEM, SHV and CTX-M β-lactamases. Plasmids were extracted by alkaline lysis methods. Genotyping of P. mirabilis strains was performed by pulsed-field gel electrophoresis (PFGE).

**Results:** ESBLs were detected in all strains by double disk synergy test. All strains were resistant to amoxycillin, gentamicin and ciprofloxacin, but susceptible to combination of ceftazidime with clavulanic acid, piperacillin/tazobactam, aztreonam, cefotaxin, imipenem and meropenem. Cephalosporins showed variable degrees of resistance; cefotaxime and ceftriaxone MICs varied between 32 and 256 mg/L, while ceftazidime MICs were slightly lower (16–256 mg/L). Cefepime had better activity with MICs varying between 8 and 64 mg/L. Three strains transferred resistance to E. coli recipient. The resistance to chloramphenicol, gentamicin and tetracycline was cotransferred alongside with ceftazidime resistance from two strains whereas sulphamethoxazole/trimethoprim resistance was transferable from one strain. A plasmid of approximately 70 kb was isolated from three representative strains. All seven strains yielded an amplicon of 853 bp with primers specific for TEM β-lactamases. Sequencing of blaTEM genes revealed TEM-52 β-lactamase. All strains have shown to possess identical PFGE patterns.

**Conclusions:** This is the first report of TEM-52 β-lactamase from Croatia. The fact that the strains were clonally related and showed similar resistance phenotypes points out that there is endemic and epidemic spread of TEM-52 producing P. mirabilis causing nosocomial infections in University Hospital Split.

**P1205 SHV-112: a novel extended-spectrum β-lactamases produced by Klebsiella pneumoniae strains isolated in Kuwait**

A. Dashti*, M. Jadaon, F. Habeeh, E. Udo (Salalahikht, KW)

**Introduction:** Extended-spectrum β-lactamases (ESBL) are plasmid mediated enzymes produced by some Enterobacteriaceae to resist β-lactam antibiotics. The number of new mutations among the ESBLs are increasing. A total of >100 mutations have been reported in the genes encoding the SHV enzymes. We hereby report a novel mutation in genes for SHV enzymes in clinical isolates of Klebsiella pneumoniae.

**Materials and Methods:** A total of 22 K. pneumoniae isolates obtained from a Kuwaiti hospital were studied. Antibiotic susceptibility testing was performed disk diffusion method. ESBL production was detected using AST-N20 card in the Vitrek system and confirmed by ESBL Etest strips (AB Biodisk). PCR was used to amplify the SHV-genes. The amplified products were sequenced by the Big Dye terminator using an automated DNA sequencer (ABI3100) to establish its relationship with previously reported SHV genes.

**Results:** All the isolates were ESBL-positive and were resistant to ceftazidime, and ceftazidime with clavulanic acid. PCR amplification yielded a product of 308 kb corresponding to the expected size of SHV gene. DNA sequencing of the SHV gene in 4 isolates yielded identical results. They revealed a mutation at position 253 from A to G resulting in a change from Aspartic acid (AAT) to Aspartine (GAT) at position 815 of the enzyme (A815G). It was assigned the unique number SHV-112, with a GeneBank accession number EU477409.

**Conclusion:** Further studies are required to assess the prevalence of A815G mutation in SHV ESBL-producing K. pneumoniae isolated in Kuwait and elsewhere.

**P1206 Novel PER-variant β-lactamase identified in Providencia rettgeri strain from the United States: report from the SENTRY Antimicrobial Surveillance Program**

M. Castanheira*, L. Deshpande, M. Loeffelholz, R. Jones (North Liberty, Galeston, US)

**Objective:** To characterise the genetic determinant responsible for the ESBL phenotype in a Providencia rettgeri bacteriaemia isolate collected in a hospital in Galveston, Texas, USA.

**Methods:** During 2007, 283 (10% of all isolates) Enterobacteriaceae isolates from SENTRY Program USA medical sites displayed elevated cephalosporin MIC values (>2 mg/L) by reference broth microdilution method. ESBL phenotypes were confirmed using Etest strips containing cefepime with and without clavulanate. ESBL genes were amplified in a multiplex PCR approach using generic primers for genes encoding PER, VER, GES, OXA-2 and OXA-10. TEM- and SHV-encoding genes were also amplified and sequenced. Primers comprising the open reading frame of blaPER were used to amplify the entire gene and amplicons were cloned into TOPO and transformed in an E. coli host for sequencing. Conjugation experiments were carried out and selected on media containing 500 mg/L of streptomycin and 8 mg/L of ceftazidime.

**Results:** P. rettgeri isolate 25–3141A was recovered in February/2007 from a bloodstream of a 65 years/old male patient hospitalised in a Texas hospital. The patient that was an inmate of the Texas State Prison for several years prior to admission for evaluation of pneumonia. He was briefly hospitalised a month earlier with diagnosis of HCV cirrhosis and ascites, when he received only one day of cefotaxime. The P. rettgeri isolate demonstrated elevated cefepime MIC (4 mg/L) and positive ESBL confirmatory test. Isolate 25–3141A also showed elevated MIC values against fluoroquinolone, trimethoprim/sulfamethoxazole and tetracycline. blaPER amplicons were detected in the multiplex PCR. Recombinant plasmids carrying the entire β-lactamase encoding gene were sequenced and the analysis of the derived aminoacid sequence showed one aminoacid alteration compared to PER-1 structure (E33G). This isolate also harboured blaTEM-1 Conjugation failed to yield colonies showing resistance to cephalosporins.
Conclusions: ESBLs, other than SHV and TEM, are becoming more common in the USA with recent reports of CTX-M-producing strains in several medical centres. In this study, we described a novel PER enzyme detected in a P. rettgeri that showed a single aminooacid difference compared to PER-1. The diversity in the β-lactamase types detected among USA isolates is rapidly increasing, changing the ESBL treatment scenario in this country.


B. Ruiz del Castillo, I. Rodriguez, S. Jahn, J. Beutlich, W. Baronwick, A. Schroter, R. Helmuth, B. Guerra *(Santander, Oviedo, ES; Berlin, DE)*

**Objective:** Molecular characterisation of antimicrobial resistance (R) in Salmonella (S.) Paratyphi B dT+ isolates showing ESBLs or AmpC phenotype originating from foods and animals in Germany.

**Methods:** Among the S. enterica isolates from animal and food origin (2003-2008) obtained in the National Reference Laboratory for Salmonella (NRL-Salm) strain collection (Berlin), all epidemiologically unrelated S. Paratyphi B dT+ isolates showing a MIC for cefotril ≥4 mg/L, were selected. A total of 12 isolates, came from six German regions, and originated from foods (8 from chicken meat, 1 minced meat, and 1 from spices) and animals (2 from broilers). Their susceptibility to 17 antimicrobial agents (including the β-lactams ampicillin and cefotril, and amoxycillin/clavulanic acid) by broth microdilution, and for an additional panel of 12 β-lactams, by disc-diffusion was tested. Molecular methods as PCR amplifications/sequencing, isoelectric focusing, PFGE with XbaI, plasmid profile analysis and Southern-hybridisation were used to characterise the resistance determinants and epidemiological relationship.

**Results:** All S. Paratyphi isolates shared a common XbaI-PFGE pattern. They showed different resistance phenotypes, and plasmid profiles, but all of them were multiresistant (more than four resistance determinants) and harboured a 2300 bp dfrA1-sat1-aadA1 class 2 integron. Six isolates carried also class 1 integrons (3 different variable regions). ESBLs were present in all isolates, whereas AmpC were found in only one of them. Four isolates carried blaCTX-M1 and 3 blaCTX-M2 genes, located on self-transferable plasmids from different sizes (80–100 kb), mainly from incompatibility group IncI1. TEM-1 variants were found in 5 isolates: 3 with blaTEM-52 and 2 with blaTEM-20 genes, also located on IncI1 plasmids. One of the blaCTX-M2 isolates also carried an AmpC blaACC1 gene. No qnrB, A, or S genes were detected.

**Conclusions:** Our results show that a wide spread S. Paratyphi B dT+ German clone previously described (Miko et al., JCM 2002), originated from foodsof avian origin, could be observed.

**[P1208]** Prevalence of extended-spectrum β-lactamases in Escherichia coli obtained from faecal samples of captive ostrich in Portugal


**Objectives:** To determine the prevalence of ESBLs in E. coli isolates obtained from faecal samples of captive ostrich from the Alentejo (Portugal), and to study the presence of other resistance genes, integrons, virulence factors and phylogenetic groups.

**Methods:** 54 faecal samples of captive ostrich were obtained and inoculated in Levine agar plates supplemented with cefotaxime (2 mg/L), and were incubated 24h at 37°C; one colony per sample with E. coli morphology was identified. Antibiotic susceptibility testing for 13 antibiotics was performed by disk-diffusion agar, and screening for ESBL production was performed (CLSI). The presence of genes encoding TEM, SHV, OXA, CTX-M and CMY β-lactamases, as well as other resistance genes (tetA, tetB, cmlA, sul1), and the characterisation of class 1 and 2 integrons was carried out by PCR and sequencing. Virulence genes (fimA, cfl1, papC, papGIII and aer) and characterisation of phylogenetic groups was determined by PCR. Clonal relationship among ESBL-positive isolates was studied by PFGE.

**Results:** ESBL-positive E. coli isolates were detected in 3 of the 54 analysed samples (5.6%), and the following β-lactamases were identified: CTX-M14 + TEM-1 (2 isolates), and CTX-M14 + TEM-52 (1 isolate). The blaCTX-M14 gene was surrounded by ISecp1 and IS903 in all cases. The three ESBL-positive isolates showed unrelated PFGE patterns, and harboured the tetA and sul1 genes (encoding tetracycline and sulphonamide resistance), as well as the aer and fimA virulence genes, being the three isolates of the B1 phylogroup.

They showed resistance to nalidixic-acid, ciprofloxacin, tetracycline, and streptomycin, in addition to β-lactams, and two of them also to chloramphenicol or trimethoprim-sulphonamide oxidation. Class 1 integrons were identified in the three ESBL-positive E. coli isolates with the following gene cassette arrangement in their variable region: aadA1 (2 isolates), and dfrA1-aadA1 (1 isolate).

**Conclusion:** The intestinal tract of captive ostrich may be a reservoir of ESBL-positive E. coli isolates. CTX-M-14, frequently detected in clinical human isolates in Portugal and Spain, especially detected in these animals, together with TEM-52.

**[P1209]** Comparative assessment of extended-spectrum β-lactamase-producing Escherichia coli contamination in different food supplies

P. Egeo *, L. López-Cerero, J. Rodríguez-Baño, M.D. Navarro, A. Pascual *(Seville, ES)*

**Introduction:** The spread of extended spectrum β-lactamases-producing E. coli (ESBLEC) in the community setting is an emerging health problem. ESBLEC has been found in livestock faeces and raw meat, especially poultry, suggesting that contaminated food could contribute to the dissemination of resistant Enterobacteriaceae in the community. High ESBLEC contaminated poultry carcasses (>95% of samples) have been detected in our area, however, few current data regarding of contamination rate of other poultry products are available.

**Objective:** This study aimed to compare the contamination rate with ESBLEC in different poultry products and salads and characterise the isolates.

**Material and Methods:** Shells from 72 prepackaged and non-packaged chicken eggs, 32 salads, and 30 samples of cooked chicken and 6 samples of pet food made of chicken meat were analyzed. Egg shells were sampled by shaking incubation in buffered peptone water and food samples were homogenised with Stomacher blender in defined volumes of peptone broth. Lactose-positive bacterial quantification was done by disc-diffusion agar, and screening for β-lactamase enzymes as PCR amplifications/sequencing, isoelectric focusing, PFGE with XbaI, plasmid profile analysis and Southern-hybridisation were used to characterise the resistance determinants and epidemiological relationship.

**Results:** One (3%) SHV-12-producing E. coli strain was detected in one salad sample. This strain belonged to B1 phylogenetic group. No ESBLEC were found in cooked poultry, pet food or eggs shells. A high lactose-positive bacteria contamination (>3 Log10 CFU/ml) was exhibited in 9.4% samples of salads, 33% of pet food and 6.7% of cooked chicken but not in shells eggs.

**Conclusions:** Raw meat may contain ESBLEC, but the effects of treatment process such cooking or dehydration could reduce the risk of transmission as well as the washing shells or sanitation procedures in the case of hens eggs. Nevertheless, fresh vegetable food can be contaminated with ESBLEC during meal preparation.
Prevalence of antimicrobial resistance and antimicrobial resistance genes among Escherichia coli from healthy volunteers and patients with urinary tract infection

S. Garlick, R. John, A. Locoring, A.P. MacGowan, M. Gal, VI. Emme

Objective: To investigate the reservoir of antibiotic resistance in commensal Escherichia coli from healthy community volunteers and how this impacts on the prevalence of antibiotic resistance in E. coli associated with urinary tract infection.

Methods: 295 E. coli isolated from healthy community volunteers and 295 E. coli isolated from clinical urine samples from the South West region of England were studied. Their susceptibility to amoxicillin, cephalaxin, ciprofloxacin, co-amoxiclav, ertapenem, gentamicin, meropenem, nitrofurantoin, tetracycline and trimethoprim was determined by agar dilution. The presence of the blaTEM, blaCTX-M-1 group, blaCTX-M-2 group, blaOXA-1 group, blavIM, aac6-ib-cr, tet(A) and tet(B) genes was determined by PCR. Statistical analysis was carried out by the chi-squared test.

Results: Isolates from urinary tract infections were more antibiotic resistant with 58.6% being resistant to at least one antibiotic and 24.7% being multi-resistant, compared to 43.4% and 9.8% for isolates from healthy volunteers. Resistance to individual agents varied between 0.3%-49.8% for urinary isolates compared to 2.4%-32.9% for commensal isolates (Table).

Among commensal isolates the frequencies of blaTEM was 19.3%, the frequency of blaOXA-1 was 0.3%, the frequency of tet(A) was 8.1%, the frequency of tet(B) was 5.8% and the frequency of aac6-ib-cr was 0.3%. blaVIM and blaCTX-M were not detected. Among urinary isolates the frequency of blaTEM was 24.9%, the frequency of blaOXA-1 was 2.5%, the frequency of blaCTX-M-1 was 0.8%, the frequency of tet(A) was 9.1%, the frequency of tet(B) was 14.1% and the frequency of aac6-ib-cr was 1.0%. blaVIM and blaCTX-M were not detected.

Conclusions: The prevalence of antimicrobial resistance and resistance genes among commensal E. coli isolates was lower than in urinary isolates, but still significant, suggesting such isolates may act as a reservoir of resistance. The occurrence of ertapenem resistance among commensal isolates is of concern.

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Characterisation of CTX-M-positive isolates of Enterobacteriaceae using real-time PCR and melting curve analysis

M. Chromát*, L. Cekanová, M. Kolár, T. Stosová, P. Sauer (Olomouc, CZ)

Objective: Bacterial resistance to β-lactam antibiotics due to extended-spectrum β-lactamase (ESBL) production is an increasing global problem. CTX-M enzymes represent a group of ESBL, often found in various isolates of enterobacteria.

Methods: During one-year period between may 2007 and 2008, rectal swabs were collected from healthy community objects living in the Olomouc Region and from patients hospitalised in the University Hospital Olomouc, Czech republic. The rectal swabs were inoculated onto the chromID ESBL selective medium. Phenotypic detection of ESBL was performed using a modified double-disc synergy test (mDDST). Molecular-genetic methods like real-time PCR with melting curve analysis, restriction fragment analysis and direct sequencing were used to confirm the phenotypic determination.

Results: A total of 579 rectal swabs from community objects were investigated. According to the positive grow on chrom ID ESBL medium and positive mDDST 7 ESBL-positive isolates of Escherichia coli were obtained. The same approach was used for 546 rectal swabs from hospitalised patients whereas 14 isolates of the same Enterobacteriaceae producing ESBL were acquired (E. coli-8 isolates, Klebsiella pneumoniae-3, Enterobacter aerogenes-1, Citrobacter freundii-2). The presence of blaCTX-M gene was detected in all ESBL-positive strains using real-time PCR technique. By use of differences between restriction patterns
and melting temperatures of the amplified products, the clusters of CTX-M enzymes (CTX-M-1 and CTX-M-9) were identified among clinical isolates. CTX-M-15 was the most widespread ESBL.

**Conclusion:** From collected data results that the occurrence of ESBL-positive bacteria in the gastrointestinal tract was confirmed in 1.2% of subjects in the community and in 2.5% of hospitalised patients. Using molecular genetic methods the appearance of CTX-M-15, CTX-M-9 like and CTX-M-1 type β-lactamases was approved.

The study was supported by the grants MSM6198959223, Czech Republic and IGA 9950-3.

**P1213** Identification of a chromosomal class A β-lactamase from *Serratia rubidaea*

**T. Naas**, A. Ergani, J. Didi, S. Lima, P. Nordmann (Le Kremlin-Bicêtre, FR)

**Background:** Within the Enterobacteriaceae species, strains of the genus *Serratia* are frequently identified in human nosocomial infections. *Serratia marcescens* and the *Serratia* liquefaciens complex are responsible of the majority of *Serratia* human infections. Human infections due to *S. rubidaea* are associated with the consumption of contaminated coconuts or vegetable salads. *S. rubidaea* has also been shown to cause sepsis and other infections in outpatients and hospitalised patients. *Serratia* spp. may be a source of difficult to treat infections since many of these strains are resistant to β-lactams mediated by the production of chromosomally-encoded β-lactamases, of either Class C (Ambler-type of *S. marcescens*), class A (FONA of *S. fonticola*) or class B (SHV-1 of an environmental *S. fonticola*). Here, we describe a novel β-lactamase from *S. rubidaea*.

**Method:** The β-lactamase from *S. rubidaea* CIP 103234T was cloned, sequenced and expressed in *E. coli*. The genetic location of the gene was determined and the enzymatic properties of purified β-lactamase analyzed.

**Results:** On a routine antibiogram *S. rubidaea* CIP 103234T reference strain displayed a weak narrow-spectrum β-lactam-resistant phenotype (reduced susceptibility to amoxicillin and ticarcillin, which was recovered by clavulanic acid). It encoded a clavulanic-acid inhibited Ambler class A β-lactamase, RUB-1, with a pl value of 6.0 and a molecular mass of ca. 29 kDa. RUB-1 had the highest percent identity with GIL-1, PLA-1, ORN-1, TEM-1, and SHV-1, 74%, 74%, 73% and 70% amino acid sequence identity, respectively. The substrate profile of the purified RUB-1 was similar to that of β-lactamases TEM-1, SHV-1 and GIL-1. The kinetic properties confirmed the penicillinase behaviour of the enzyme. The blaRUB-1 gene was chromosomally-located as revealed by I-CeuI-experiments and no gene homologous to β-lactamase expression in *S. marcescens* was determined and the enzymatic properties of purified RUB-1 were characterized.

**Conclusion:** In this study we have characterised a new enzyme, SHV-84, that exhibits an unusual resistant profile to the amoxicillin-clavulanic acid combination. Unlike SHV-72, another IR-SHV harbouring the same extended-spectrum β-lactam resistance phenotype (blaCMY-2), the SHV-84 has a lower affinity to penicillins than SHV-1 and a decreased catalytic activity for these antibiotics. On the other hand, the SHV-84 is less susceptible to clavulanic acid than SHV-72. In conclusion, this study underlines the role of the Lys234Arg substitution in the resistance to clavulanic acid.

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**P1215** Sporadic occurrence of CMY-2 producing multidrug-resistant *Escherichia coli* of clonal complex (CC)48, CC38 and CC131 in Norway, 2003–2007. Indications for globally disseminated clones?

**U. Nasci***, B. Haldorsen, G.S. Simonsen, A. Sandsford (Trondheim, NO)

**Objectives:** The spread of plasmid-mediated AmpC β-lactamases (PABLs) among *Escherichia coli* worldwide is worrisome. Population analysis of PABL-producing *E. coli* are lacking. In this study we collected clinical isolates of *E. coli* with reduced susceptibility to oximino-cephalosporins without clavulanic acid synergy from Norwegian diagnostic laboratories in 2003–2007 for molecular characterisation.

**Methods:** Isolates with a positive boronic acid test (n = 276) were examined for plasmid-mediated ampC genes using multiplex-PCR. Positive isolates (n = 38) were further examined by blacym-PCR sequencing typing, antimicrobial susceptibility testing for β-lactam and non-β-lactam antibiotics by Etest and Vitek2, respectively. Moreover, characterisation of the genetic environment of blacym, phylogenetic grouping, XbaI-PFGE, MLST, plasmid profiling, PCR based replicon typing and plasmid transfer was performed.

**Results:** PABL-positive isolates (n = 38) were typed as blacym2 (n = 35), blacmy7 (n = 1) and bladhA1 (n = 2), from out-patients (n = 23) and in-patients (n = 15) expressing moderate to high MICs for all β-lactam substrates, except cephrpine and carbapenems. All isolates were co-resistant to trimethoprim-sulphamethoxazole and 58% expressed multidrug-resistance. Thirty-two (91%) blacym2 and one blacmy7 were linked to IS1218 upstream and one bladhA1 (50%) was linked to qacEAl1 sul1 upstream and downstream. Twenty isolates (53%) were of putative virulent phylogenetic groups B2 and D. Thirty-three XbaI-PFGE-types including three small clusters were observed. Twenty-five sequence types (STs) were identified. The dominant STs were related to clonal complex (CC) 38 (n = 7), CC48 (n = 5) and CC131 (n = 4). Plasmid profiling revealed 1–4 plasmids of 50–250 kb per isolate and 11 different replicons in 37/38 isolates. Blacym2 was seen carried on transferable multiple-replicon plasmids dominated by H1 (n = 12), HII (n = 10) and A/C (n = 7). Interestingly, Southern blot hybridisation indicated chromosomal integration of blacmy-2 in 9 isolates belonging to CC448 and CC38.
ESBLs, AmpCs & others in Enterobacteriaceae: genes, plasmids & clones – part 2

S333

Conclusion: CMY-2 is the dominant PABL among Norwegian E. coli isolates. It is strongly associated with ISEcp1 upstream and identified on multiple-locus MLST (IncI1, IncC/A or IncFII) transferable plasmids. The occurrence of PABLS in Norway is sporadic and is probably linked to globally dispersed urapathogenic strains of CC38, CC448 and ST-131 of which the latter has been associated with the spread of CTX-M-15.

P1216 Acquisition of a plasmid carrying blaCMY-2 by the established blaOXA-30-producing Salmonella Typhimurium Iberian clone

P. Antunes*, L. Peixe (Porto, PT)

Objectives: Multidrug-resistant (MDR) Salmonella is emerging worldwide, with increasing involvement of particular clones in human infections. Given the importance of cephalosporins in therapeutics, our goal was to characterise mobile genetic elements associated to β-lactams resistance in a predominant MDR Salmonella Typhimurium clone causing foodborne infections in Portugal and Spain over years.

Methods: We analysed 46 blaOXA-30-producing Salmonella Typhimurium isolates belonging to a previously described Iberian clone obtained from human clinical infections, food products and environment in different regions of Portugal (2002–2008). The isolates were examined for susceptibility to antimicrobial agents and β-lactamase production. Detection of resistance genes and integrons was done by PCR. Class 1 integrons were characterised by PCR, RFLP (TaqI) and sequencing. Clonality was established by PFGE (XbaI). Plasmid analysis included conjugation assays, extraction of DNA and sequencing, determination of size (S1-PFGE) and content (incompatibility groups by rep-PCR typing, hybridisation and sequencing). Location of integron and β-lactamasewas performed by hybridisation of I-CeuI/S1-PFGE.

Results: The isolates were MDR (predominant phenotype: amoxicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline) and shown to be resistant to amoxicillin, clavulanic acid and ticarcillin. Screening by PCR of resistance genes blaCMY-2 and blaOXA-30 was performed. The isolates were high-level ESBL producers (5.1 ml/L) and were phenotypically resistant to cephalosporins (16−32 mg/L). The ESBL combined disk test did not showed synergy between clavulanic acid and indicator cephalosporins, while the AmpC disk test proved to be positive.

Conclusion: The isolates were highly resistant to cephalosporins (>256 mg/L), cefotaxime (64 mg/L), gentamicin (128 mg/L), and amikacin (>256 mg/L) and ciprofloxacin (>32 mg/L) and moderately resistant to cefoxitin (16−32 mg/L). The ESBL combined disk test did not showed synergy between clavulanic acid and indicator cephalosporins, while the AmpC disk test proved to be positive. According to the PFGE analysis the strains showed identical macrorestriction profiles. The strains carried a large non-transferable plasmid of app. 140 kb in size which was cured from their hosts. The plasmid cured strains became susceptible to cefazidime (0.25 mg/L), cefotaxime (0.032 mg/L), gentamicin (1 mg/L) and amikacin (4 mg/L). By PCR screening, the strains were found to be positive for blaACC and blaTEM. Sequence analysis of β-lactamase genes detected blaACC-4 and blaTEM-1 in both isolates.

Conclusions: To our best knowledge this is the first report of plasmid-mediated AmpC-type β-lactamase in Hungary. The MICs of cefazidime, cefotaxime, cefepime, cefotin, imipenem, gentamicin, amikacin and ciprofloxacine were determined by the E-test (AB Biodisk). The phenotypic investigations of mechanism of resistance to cephalosporins were performed by ESBL combined disk test (MAST) and AmpC disk test. The isolates were examined for the presence of bla SHV, blaTEM, blaCTX-M, blaMOX, blaCMY, blaLAT, blallback, blaDHA, blaACC, blaMIR, blaACT, blaflox by PCR and the amplified genes were sequenced. Conjugation and plasmid curing experiments were carried out also. Furthermore typing with pulsed field gel electrophoresis was performed.

Results: The isolates were highly resistant to cephalosporins (>256 mg/L), cefotaxime (64 mg/L), gentamicin (128 mg/L), amikacin (>256 mg/L) and ciprofloxacin (32 mg/L) and moderately resistant to cefoxitin (16−32 mg/L). The ESBL combined disk test did not showed synergy between clavulamic acid and indicator cephalosporins, while the AmpC disk test proved to be positive.

According to the PFGE analysis the strains showed identical macrorestriction profiles. The strains carried a large non-transferable plasmid of app. 140 kb in size which was cured from their host. The plasmid cured strains became susceptible to cefazidime (0.25 mg/L), cefotaxime (0.032 mg/L), gentamicin (1 mg/L) and amikacin (4 mg/L). By PCR screening, the strains were found to be positive for blaACC and blaTEM. Sequence analysis of β-lactamase genes detected blaACC-4 and blaTEM-1 in both isolates.

Conclusions: To our best knowledge this is the first report of plasmid-mediated AmpC-type β-lactamase in Hungary and blaACC-4 in Europe as well as the first blaACC in Proteus mirabilis.
associated to resistance or reduced susceptibility to third-generation cephalosporins and penicillin plus β-lactamase inhibitor combinations, and can also result in reduced susceptibility to carbapenems in presence of decreased outer membrane permeability. In this work we report on a long-lasting and complex outbreak caused by multidrug-resistant (MDR) Enterobacteriaceae producing the FOX-7 β-lactamase in an NICU setting.

**Methods:** Antimicrobial susceptibility was determined by disk diffusion and broth dilution methods. Clonal relatedness was investigated by PFGE of XbaI-digested genomic DNA. β-lactamase genes were investigated by PCR and sequencing.

**Results:** MDR enterobacteria of various species (mostly Klebsiella pneumoniae but also Klebsiella oxytoca and Pantoea agglomerans) resistant to third-generation cephalosporins but not producing extended-spectrum β-lactamases (ESBLs) were isolated from NICU patients of Sienna University Hospital from January to November 2008. Overall, the outbreak involved 27 patients, yielding 23 non replicate isolates of K. pneumoniae. Enterobacteriaceae in an NICU setting, which underscores the potential relevance of these emerging resistance determinants also in this peculiar setting. Plasmid spread (under investigation) apparently played a dominant role in evolution of the outbreak.

**Conclusions:** Sporadic cases and outbreaks of pACBL-producing Enterobacteriaceae have been reported in various settings. To our best knowledge, this is the first description of a large, complex (multiclonal and heterospecific) and prolonged outbreak of pACBL-producing enterobacteria in an NICU setting, which underscores the potential relevance of these emerging resistance determinants also in this peculiar setting. Plasmid spread (under investigation) apparently played a dominant role in evolution of the outbreak.

**Objective:** To characterise an unusual ESBL phenotype (“cefeipimase”) observed in 18 isolates of S. Typhimurium. To characterise an unusual ESBL phenotype (“cefeipimase”) observed in 18 isolates of S. Typhimurium.

**Methods:** Antimicrobial susceptibility testing against 21 antimicrobial agents was performed by CLSI disk diffusion methods. Isolates were evaluated for the presence of Class I and Class II integrons and prolonged outbreak of pACBL-producing enterobacteria in an NICU setting, which underscores the potential relevance of these emerging resistance determinants also in this peculiar setting. Plasmid spread (under investigation) apparently played a dominant role in evolution of the outbreak.

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**Central nervous system infections**

**Objective:** We compared clinical characteristics and outcome in adults with community-acquired pneumococcal meningitis before and after the introduction of adjunctive dexamethasone (DXM) therapy. We compared clinical characteristics and outcome in adults with community-acquired pneumococcal meningitis before and after the introduction of adjunctive dexamethasone (DXM) therapy. **Methods:** We compared two Dutch prospective nationwide cohort studies on community-acquired pneumococcal meningitis. We selected patients with cerebrospinal fluid (CSF) culture proven meningitis: 352 consecutive patients were enrolled in the period 1998–2002 before routine DXM therapy was introduced, and 279 patients were enrolled in the period 2006–2008 after guidelines recommended routine use of DXM in the Netherlands. Outcome was graded with the Glasgow Outcome Scale and a score of 4 or less was considered unfavourable. **Results:** Baseline characteristics were similar between both cohorts: mean age was 58 and 59 years for cohort I and II, respectively (p = 0.74); median score on Glasgow Coma Scale 10 and 10 (p = 0.73); hemiparesis 12% and 12% (p = 0.57); heart rate 100 and 100 (p = 0.71); and median thrombocytopenia count 199 and 197 per L (p = 0.97). DXM was administered in 59 (17%) patients in cohort I and in 252 (92%) patients in cohort II (p < 0.001). DXM was started with or before the first dose of antibiotics in 1% and 82% respectively (p < 0.001). In cohort II, 217 (80%) received a 4 day regimen of DXM started before or with first dose of antibiotics as recommended in the guidelines. The proportions of patients with systemic complications were similar between cohorts (38% vs 42%; p = 0.29), whereas neurologic complications were less likely to occur in cohort II (78% vs 68%; p = 0.03). The proportions of patients with unfavourable outcome (50% vs 39%; p = 0.005), hearing loss (22% vs 11%; p < 0.001), or death (30% vs 22%; p = 0.02) were significantly smaller in cohort II as compared to cohort I.

**Conclusion:** 1) Adjunctive dexamethasone has been implemented for pneumococcal meningitis in the Netherlands. 2) Implementation of adjunctive dexamethasone had improved outcome in pneumococcal meningitis.
imaging (MRI) of the brain (41 patients), electroencephalography (EEG, 33 patients), and examination by an ophthalmologist. The blood, CSF, and urine samples were analyzed for PUUV using reverse-transcription PCR (RT-PCR). The CSF samples were analyzed for white blood cell count, glucose, and protein level. **Results:** Majority of the patients (50 patients, 86.2%) suffered from symptoms typical of CNS involvement (headache, vomiting, and dizziness). MRI of brain showed abnormalities of any degree in 23 of 41 patients and seven of them involved hypophysis. We received 42 CSF samples and 21 of them had abnormal protein level, and/or white blood cell count. The PCR reaction was positive for PUUV in one CSF sample only. The EEG findings were normal in all patients. **Conclusion:** CNS-related symptoms such as headache, dizziness and vomiting were common findings in acute NE. A high number of findings in brain MRI were recorded and their clinical significance requires further evaluation. Signs of inflammation were common in the CSF although only one CSF sample was positive for PUUV. Because of severe thrombocytopenia, the samples were most likely collected too late during the course of the illness to recover PUUV from the CSF. Based on our results, we conclude that acute NE caused by PUUV affects the CNS commonly.

**Conclusion:**

CNS-related symptoms such as headache, dizziness and vomiting were common findings in acute NE. A high number of findings in brain MRI were recorded and their clinical significance requires further evaluation. Signs of inflammation were common in the CSF although only one CSF sample was positive for PUUV. Because of severe thrombocytopenia, the samples were most likely collected too late during the course of the illness to recover PUUV from the CSF. Based on our results, we conclude that acute NE caused by PUUV affects the CNS commonly.

**P1223 In vitro comparison of bacterial permeability of different epidural catheter filters**

A. Sener*, Y. Erkin, A. Tasdogen, E. Dokumucu, Z. Elar (Izmir, Canakkale, TR)

**Objectives:** The rate of infection occurrence is approximately 5.4% during epidural analgesia/anaesthesia administration. Requirement for bacteria filters to reduce the infection risk is one of the most debated topics. Conflicting results have been reported for the effectiveness of different filters for short and long term use with the epidural catheters. **Methods:** We aimed to compare the effectiveness of different filters (Portex™, Rusch™) in filtering bacteria Staphylococcus aureus (ATCC 25923) and Pseudomonas aeruginosa (ATCC 27853) using Patient Controlled Analgesia (PCA) pump in an in vitro model and Scanning Electron Microscope (SEM) was used to prove filtration of bacteria. Bacteria suspensions (0.5 Mcf of S. aureus and P. aeruginosa) are prepared in 250 mL isotonic NaCl. Two different bacteria suspensions and control groups (sterile 250 mL isotonic NaCl) are filtered using 2 different types of filters (n = 60) for 48 hours at a infusion rate of 5 mL/h by using PCA pump and collected in a sterile bottle. Bacterial colonies were counted from the samples taken from the bottles (n = 60) and filters (n = 60). The results were compared with Mann-Whitney U test. A p value of <0.05 is accepted significant.

**Results:**

Structures of the membranes in the filters and bacterial adherence were investigated using SEM and recorded. We observed that the structure of Rusch™ filters were fibrous and scattered, Portex™ filters were granular and compact. According to colony numbers counted from the bottles that represents the epidural space in clinics we established that Portex™ filters are 18 times more effective than Rusch™ filters (p = 0.0001). The presence of some bacteria in the solutions in the bottles shows that neither of them were 100% safe.

**Conclusion:**

As a conclusion; although they are not completely confidential the filters can be used as a barrier to prevent occurrence of infection. We think that all the filters should be compared with a similar methodology before they are used in routine clinical practice.

**P1224 High doses of cefotaxime for cephalosporin-resistant pneumococcal meningitis in adults. A 19-year experience**

I. Cabello*, C. Cabellós, F. Tubau, R. Verdalguer, J. Liñares, F. Guasol, P. Viladrich (L’Hospitalet de Llobregat, ES)

**Objective:** To evaluate the efficacy of high-dose cefotaxime (CTX) in the therapy of cephalosporin-resistant pneumococcal meningitis in adults. **Methods:** Patients with suspected pneumococcal meningitis were empirically treated in the emergency room with both high-dose CTX (300–350 mg/kg/day – maximum 24 g/day) and adjuvant therapy with dexamethasone, manitol and phenytoin. Once the S. pneumoniae susceptibility was known, high-dose CTX schedule was kept if a CTX resistant strain (MIC ≥ 1 mcg/mL) was isolated, or was changed to ceftriaxone (4 g a day) in susceptible cases (MIC < 1 mcg/mL). MICs were determined by microdilution method and confirmed by E-test when available. Cases with negative cultures because of previous β-lactamic antibiotic therapy but CSF positive antigen were considered as susceptible and treated with ceftriaxone. The duration of antibiotic therapy was 10 days. **Results:** Between 1990 and 2008, 93 patients (58 M/35 F), mean age 62.2±15.3 (23–88), with ultimately proved pneumococcal meningitis by positive CSF culture or positive CSF pneumococcal antigen (BINAX), were empirically treated with high-dose CTX. 79 cases (85%) resulted CTX-susceptible and 14 (15%) CTX-resistant – 12 cases MIC 1 mcg/mL (85.7%) and 2 cases 2 mcg/mL (14.2%). High-dose CTX therapy was kept in all CTX-resistant cases and in 4 CTX-susceptible cases with MICs of 0.5 mcg/mL. Early adjuvant therapy was similar in both groups. Among CTX resistant cases, one case was considered a clinical but not bacteriological failure in the fifth day and then treated and cured by adding vancomycin without sequelae. Mortality was 5 patients (35%), all of them were patients with strains with MIC ≥ 1 mcg/mL, 2 cases due to early neurological complications and 3 cases due to late complications after finishing meningitis therapy. Sequelae were present in 1 case and there were no relapses. No adverse events attributable to high-dose CTX were observed – except for phlebitis in 2 cases (14%). In no cases intolerance caused any change in therapy. **Conclusion:** High-dose cefotaxime constitutes an appropriate and safe therapy for the majority of cases of adult pneumococcal meningitis due to S. pneumoniae with MIC up to 2 mcg/mL. However, a close surveillance should be always taken and vancomycin should be added in case of no rapid improvement.

**P1225 Models of predicting the risk of brain herniation in bacterial meningitis**

C.N. Meyer, S. Augustesen* (Roskilde, Holbæk, DK)

**Objectives:** The purpose of our study was to evaluate models predicting a risk of brain herniation among patients with verified acute bacterial meningitis. These models are used in daily practise to indicate when to do a head CT scanning before lumbar puncture; ideally thus preventing brain herniation caused by accelerated brain shift following the spinal tap. **Methods:** The predictive models originated from North-American, British, and the Dutch guidelines (Tonkel-AR 2004, BIS algoritme 2004 (similar to Fitch-MT 2007), van Creveld-H 2002). On a national basis, unselected patients with microbiologically and clinically verified acute bacterial meningitis from 47 hospitals during 2 years (n = 320) were included. Survival data and clinical data from the medical records
were evaluated retrospectively. Two-tailed \( p < 0.05 \) indicated a significant difference.

**Results:** In 5 patients, brain herniation occurred. Among the 316 patients with available sufficient clinical data, 85% fulfilled the North-American model for early CT-scanning (before lumbar puncture), but in practice only 19% (52/269) fulfilling the criteria were handled accordingly. The British model was fulfilled by 33% of the patients, but only 32% of these (35/111) were handled accordingly. And very similar numbers were found in the Dutch model (31% and 34% of these, respectively).

Antibiotics were given before lumbar puncture in 30% (6/20) of patients suspected of meningitis, who were sent to early (before lumbar puncture) CT scanning.

**Conclusions:** According to North-American guidelines, most of the patients with meningitis should have had a CT-scan done before lumbar puncture, though according to Dutch or British guidelines this was recommended in much fewer patients. In a majority of suspected meningitis patients, timely therapy before early CT-scan was not given. Unnecessary CT scanning may still delay relevant treatment and thus have detrimental effect in acute bacterial meningitis.

**P1226** Brain herniation and the use of CT-scanning in acute bacterial meningitis
C.N. Meyer*, S. Augustesen (Roskilde, Holbæk, DK)

**Objectives:** The purpose of our study was to evaluate the circumstances surrounding brain herniation among patients with verified acute bacterial meningitis, to analyse the use of early (before lumbar puncture) head CT-scanning, and to evaluate whether the guidelines concerning early initiation of therapy (before CT-scanning) were followed.

**Methods:** On a national basis, unselected patients with microbiologically and clinically verified acute bacterial meningitis from 47 hospitals during 2 years (n = 320) were included. Survival data and clinical data from the medical records were evaluated retrospectively. Two-tailed \( p < 0.05 \) indicated a significant difference.

**Results:** Among 320 patients with acute bacterial meningitis, a total of 161 CT-scans were performed, 57 CT scans were done before lumbar puncture, and initial considerations of bacterial meningitis were documented in 20 of these. In the other 37 cases, only other diagnostic considerations than meningitis were documented. Five of 320 patients (1.6%) had brain herniation. Brain herniation occurred after spinal tap in 2 patients with no signs of brain shift on the recent CT-scan, and in 3 patients who had lumbar puncture done before the accomplishment of a CT scan which showed signs of incarceration in 2 cases. In 4 of the 5 patients, cerebral oedema was described, and 1 CT scan was described as normal. Though, cerebral oedema was described in 14 other patient without brain herniation (specificity [52–14]/52=0.73). In the 20 early CT patients suspected of meningitis, antibiotic therapy was given before CT-scan or lumbar puncture in 30% (6/20). Median time from admission to first relevant antibiotic dose given among the early CT scanned patients (225 minutes) differed significantly from the never scanned patients (75 minutes, \( p < 0.001 \)).

**Conclusions:** Brain herniation could not safely be predicted by CT-scanning alone, as the sensitivity was rather low (2/5). The finding of cerebral oedema may be seen as a radiological red flag for brain herniation with a rather low specificity (0.73). When bacterial meningitis was suspected, the clinical guidelines were not followed satisfactorily concerning swiftly administration of therapy without awaiting the result of the CT scan.

**P1227** Laboratory predictors of meningitis in scrub typhus
D.M. Kim* (Gwang-Joo, KR)

**Background:** Scrub typhus is a febrile illness caused by Orientia tsutsugamushi. We experienced cases of scrub typhus accompanying meningitis, one of the rare but life-threatening complications, and attempted to find out laboratory predictors of the meningitis.

**Methods:** One hundred and fifty patients who were diagnosed with scrub typhus at Chosun University Hospital between 2004 and 2006 were included in this study. We performed CSF analysis on scrub typhus patients with altered mentation and severe headache, and divided the patients into the meningitis and non-meningitis groups.

**Results:** Altered mentation (58.3%) and nuchal rigidity (61.5%) occurred more frequently in the meningitis group than in the non-meningitis group (\( p < 0.01 \)). Albumin concentration was lower in the meningitis group (3.24 g/dl ±0.45) than in the non-meningitis group (3.6 g/dl ±0.6) (\( p = 0.029 \)). No other significant laboratory findings were not found.

**Conclusions:** These results suggest that serum albumin level in scrub typhus patients with nuchal rigidity and altered mentation may be a simple and useful predictor of meningitis.

**P1228** Meningococcal meningitis: a review of laboratory features during an 8-year period in a general hospital

**Objectives:** The aim of the study was to evaluate retrospectively the laboratory features of suspected meningococcal meningitis in patients admitted to our hospital.

**Methods:** During a 8-year period (2001–2008) in total 1342 CSF specimens from 957 patients were analyzed. Acute meningococcal meningitis has being diagnosed in 29 patients (17 children, 12 adults). The diagnosis was established on CSF cytochemical characteristics, positive Gram stain, CRP of blood, CSF and blood culture, detection of antigens and multiplex PCR for CSF and blood samples. The antimicrobial sensitivity was determined by MIC (E-test AB Biodisk, Sweden). PCR and E-test were determined by the National Meningitis Reference Laboratory.

**Results:** From the 29 patients with positive CSF samples by PCR, 12 were positive by CSF culture too, while 17 patients were CSF culture negative. 25/29 were PCR positive in both CSF and peripheral blood samples. 22/29 strains, half of them concerning adults, were classified as serogroup B, with B:4P.114 as the predominant phenotype. Two of the 29 were classified as serogroup C, 1/29 as serogroup A (polyvalent group) and 4/29 as non-groupable. About 1/3 of the patients had also positive blood culture. All strains were sensitive to penicillin, rifampicin, cefaclor, ciprofloxacin, ceftriaxone and chloramphenicol.

**Conclusions:**
1. Bacteriological culture remains a useful tool in the diagnosis of bacterial meningitis, the detection of Neisseria meningitidis and for the determination of susceptibility patterns.
2. However, multiplex PCR is particulary useful in patients who are culture(−) and/or microscopy(−), due to their prior antibiotic treatment.
3. A dramatic decrease in serogroup C is observed in Greece, prior to the introduction of the vaccine in 2000.
4. Serogroup B is still predominant, with an emerging shift to adult population.

**P1229** Listeria monocytogenes meningitis: clinical characteristics and outcomes
O. Dzupova*, J. Benes, S. Poliukova (Prague, CZ)

**Objectives:** Listeria monocytogenes is an important cause of acute bacterial meningitis. Compromised cellular immunity and elderly are considered the most important predisposing factors. Listeria meningitis is supposed to have some distinct clinical and laboratory characteristics in comparison to meningitis caused by other bacteria. The aim of our study was to find out the frequency and to evaluate predisposing factors, clinical and laboratory features and outcome of Listeria meningitis.

**Methods:** A longitudinal prospective study of adult patients with community-acquired acute bacterial meningitis was carried out at the
Fibronectin stimulates microglia to phagocytose bacteria in a dose-dependent manner. This approach could improve the brain resistance of immunocompromised patients against infections caused by E. coli.

**Introduction:** A multidrug-resistant Enterobacter hormaechei strain (EHOS) caused a nationwide outbreak in The Netherlands. Previous studies showed that this EHOS contained a High Pathogenicity Island (HPI), encoding a highly efficient iron uptake system and a conjugative plasmid with multiple resistance genes. It was also shown that the multidrug resistance phenotype was not enough to cause the strain to become highly epidemic. To identify other features that may have contributed to the epidemic behaviour of the EHOS the chromosome of the EHOS was sequenced. In addition the mobility of the HPI was investigated.

**Methods:** DNA of a representative isolate was sequenced using 454 pyrophosphate sequencing technology with 24-fold coverage by Roche Applied Sciences. Resulting reads were assembled in contigs using the 454 Newbler assembler. By PCR and Sanger sequencing the number of an epidemic Enterobacter hormaechei

**A. Paauw, M.A. Leeverstein-van Hall**, J. Verwoerd, A.C. Fluitt (Utrecht, NL)

**Objectives:** Infections caused by Enterobacter coli are common in the clinical setting and are still associated with high rates of mortality and long term sequelae despite antimicrobial therapy. Parenchymal microglia are one of the effective defence systems in the brain to remove invading bacteria contributing to the resistance of the brain. Microglia express Toll-like receptors (TLRs) that recognize invading pathogens as well as endogenous proteins at non-physiological concentrations such as fibronectin (Fn). Here, we hypothesized that the endogenous TLR4 ligand Fn might protect immunocompromised patients against infections by increasing the ability of microglial cells to phagocytose E. coli.

**Methods:** Primary cultures of mouse microglia were exposed to increasing concentrations of Fn (10, 50 or 100 mg/l) for 24 h. A control group of unstimulated cells was included in all experiments. After stimulation, supernatants were collected and stored at −80°C until measurement of cyto-/chemokine levels. Then, microglial cultures were challenged with either live E. coli DH5alpha or E. coli K1 at a ratio of 100 bacteria per cell. Phagocytosis was left to proceed for 30 and 90 min. For phagocytosis inhibition studies, cytochalasin D (CD) was used at 10 μM. After bacterial exposure, microglial cultures were washed and lyzed with distilled water. Viable intracellular bacteria were enumerated by quantitative plating of serial 10-fold dilutions. ANOVA (followed by Bonferroni’s multiple comparisons test) was performed to analyse differences between groups (n ≥ 12); p < 0.05 was considered statistically significant.

**Results:** The supernatants of unstimulated cells were devoid of measurable amounts of cyto-/chemokines. Unstimulated microglia ingested bacteria at a low rate. The endogenous TLR4-ligand Fn stimulated murine microglial cultures in a dose-dependent manner to secrete pro-inflammatory compounds and increase their ability to phagocytose E. coli DH5alpha (p < 0.05 after 30 and 90 min) and E. coli K1 (p < 0.05 after 90 min). CD blocked the entry of E. coli strains by >90%.

**Conclusion:** Fibronectin stimulates microglia to phagocytose bacteria in a dose-dependent manner. This approach could improve the brain resistance of immunocompromised patients against infections caused by E. coli.

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**Pathogenesis of infections caused by Gram-negative bacteria and mycobacteria**

**P1230** Fibronectin stimulates Escherichia coli phagocytosis by microglial cells

S. Ribes*, S. Ebert, T. Regen, N. Adam, S. Bunkowski, U.K. Hanisch, S. Hammerschmidt, R. Nau (Gottingen, Greifswald, DE)

**Objectives:** Infections caused by Escherichia coli are common in the clinical setting and are still associated with high rates of mortality and long term sequelae despite antimicrobial therapy. Parenchymal microglia are one of the effective defence systems in the brain to remove invading bacteria contributing to the resistance of the brain. Microglia express Toll-like receptors (TLRs) that recognize invading pathogens as well as endogenous proteins at non-physiological concentrations such as fibronectin (Fn). Here, we hypothesized that the endogenous TLR4 ligand Fn might protect immunocompromised patients against infections by increasing the ability of microglial cells to phagocytose E. coli.

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**Conclusion:** Fibronectin stimulates microglia to phagocytose bacteria in a dose-dependent manner. This approach could improve the brain resistance of immunocompromised patients against infections caused by E. coli.
The siderophore yersiniabactin produced by the multidrug-resistant uptake, which is essential for bacterial growth. The genetic composition of HPI. The siderophore yersiniabactin encoded by the HPI facilitates iron reduction of the ROS response of PMNs was observed. Iron-saturated environment. The use of hemin or transferrin as a main iron source did not contribute to their strong colonising capacity in the human colon and it may not be regarded as a fitness island, on the contrary it is more likely to be an actual pathogenicity island.

**[P1232]** The siderophore yersiniabactin produced by the high-pathogenicity island of Enterobacteriaceae reduces the oxidative stress response of polymorphonuclear leukocytes

A. Paauw, M.A. Leeverstein-van Hall*, J. Verhoef, A.C. Fluit (Utrecht, NL)

**Introduction:** Yersinia spp. or Escherichia coli containing the High Pathogenicity Island (HPI) are more virulent than strains lacking the HPI. The siderophore yersiniabactin encoded by the HPI facilitates iron uptake, which is essential for bacterial growth. The genetic composition of the HPI may vary between isolates. An HPI was detected in a multidrug-resistant Enterobacter hormaechei outbreak strain (EHOS) that caused invasive infections in over 100 patients throughout The Netherlands.

The aim of this study was to test the hypothesis that yersiniabactin inhibits the innate immune system at the site of infection as a result of the competitive binding for iron between yersiniabactin, produced by HPI-positive bacteria and lactoferrin (LF), produced by polymorphonuclear leukocytes (PMNs). PMNs require LF-bound iron to form radicals and peroxides to kill phagocytosed pathogens. Furthermore the genetic structure of the EHOS-HPI was characterised.

**Methods:** An HPI-knockout, unable to produce yersiniabactin, was created. Growth experiments with different iron sources were performed using the HPI-knockout and the wild-type strain. The reactive oxygen species (ROS) response of stimulated PMNs incubated with and without yersiniabactin was measured with luminol. The HPI structure was determined by PCR.

**Results:** The production of yersiniabactin improved the ability of the EHOS to obtain iron from saturated LF in an iron-depleted environment. The use of hemin or transferrin as main iron source did not result in growth differences. A yersiniabactin concentration dependent reduction of the ROS response of PMNs was observed. Iron-saturated yersiniabactin showed no reduced ROS response. The reduction of the ROS response by yersiniabactin was less in the presence of increased LF or transferrin concentrations. The HPI of the EHOS had a 3'-end that was identical to the ICE of E. coli ECO31 (HPI-ICEEc1), with the exception that in the EHOS the 3'-end lacked the trapA gene. This new variant was designated HPI-ICEEc1.

**Conclusions:** The results support the hypothesis that expression of the HPI inhibits the innate immune system by reducing the availability of iron for the PMN as a result of the competitive binding for iron by the HPI-encoded yersiniabactin and host-encoded LF at the site of infection.

The EHOS contained a new variant of the HPI, designated HPI-ICEEc1, which showed a high similarity to HPI-ICEEc1.

**[P1233]** The genotypos producing E. coli in the intestinal microbiota; a new view on commensalism

FL. Nowrouzian* (Gothenburg, SE)

**Objective:** The gut microbiota contains a large amount of bioactive substances and it is likely that intestinal microbiota plays a role in the pathogenesis of bowel diseases that may be contributing to the increased risk of cancer development. E. coli is a ubiquitous member of the gut microbiota and segregates into four major phylogenetic groups, termed A, B1, D and B2.

Group B2 strains cause the most extra-intestinal infections, and have an increased capacity to persist in the colonic microbiota. Virulence factor of these strains may be located in particular regions such as the pks island carried by certain phylogenetic B2 strains. Contact with E. coli strains expressing the pks island allows DNA double-strand breaks leading to the cell death. We have studied whether the pks island is of ecological importance for E. coli in the intestinal microbiota, and/or is increased in strains capable of long-term persistence.

**Methods:** E. coli strains isolated from colonised microbiota of 70 Swedish infants was followed longitudinally over the first year of life. Overall, 143 E. coli strains of which 57 resident strains persisting in the intestine for at least three weeks and 16 transient strains were screened for the carriage of the pks island using a duplex PCR assay (polymerase chain reaction). These strains were previously tested for the phylogenetic origin.

**Results:** Totally, thirty-five percent of E. coli strains (50/143) carried the pks island of which all belonged to the phylogenetic group B2. In fact, more than two-thirds of B2 E. coli strains (50/70) were positive for the pks island. Among strains resident in the microflora, 46% carried the pks island, while this was true for 19% of the transient strains (p = 0.08).

**Conclusion:** A majority of group B2 strains from intestinal microbiota of Swedish infants carried the pks island. There was no significant difference concerning the carriage of the pks island between resident and transient strains. Thus, it indicates that this bacterial trait carried exclusively by group B2 E. coli strains does not contribute to their strong colonising capacity in the human colon and it may not be regarded as a fitness island, on the contrary it is more likely to be an actual pathogenicity island.

**[P1234]** The influence of some probiotic cultures supernatants on the growth rate and virulence expression of several selected enteroaggregative E. coli clinical strains

V. Lazur, Y. Miyazaki, T. Hanawa, M.C. Chifiriuc, L.M. Ditu, L. Marutescu*, C. Bleotu, S. Kamiya (Bucharest, RO; Tokyo, JP)

**Purpose:** To investigate the in vitro antimicrobial activity of three lactic acid bacterial (LAB) strains supernatants obtained from cultures of Bifidobacterium breve, Enterococcus faecium and Lactobacillus casei of human/animal origin against enteroaggregative Escherichia coli (EAggEC) strains isolated from diarrheal stools.

**Material and Methods:** The qualitative and quantitative study of the influence of LAB supernatants on the adherence capacity of the pathogenic EAggEC strains to the cellular substratum was performed by Cravito adapted method, in 2 variants: the simultaneous addition of LAB supernatants and the microbial suspensions on the cellular substratum and respectively, the addition of the LAB supernatants after 2 hrs incubation of microbial strains and the cellular substratum. The influence of LAB supernatants on biofilm development on inert substratum was assessed by the rapid microtiter plate method. The cytotoxicity of the tested supernatants was assessed using a Cell Counting kit.

**Results:** Our in vitro studies are demonstrating that the selected supernatants, when added simultaneously are generally opposing to the adherence to the cellular substratum by the enteroaggregative strains
(10–1000 fold decrease of the viable cells and adherence indexes). When added after the preadherence period, the supernatants did not change the adherence indexes of the EAggEC strains, but induced slight changes in the adherence pattern, reducing the frequency and size of bacterial aggregates. Only in few cases, the bacterial growth rate was slightly increased or sustained by the probiotic supernatants; a possible explanation could be the fact that in this study there were used supernatants of 24h fresh cultures, which are probably still containing some nutrients and probably also other growth factors, not only inhibitory substances, for the assay of antimicrobial activities of the probiotic supernatants, 48 hrs cultures being more appropriate.

**Conclusion:** Our results are demonstrating that soluble probiotic metabolites accumulated in culture supernatants may interfere with the first step of adherence and colonisation of the cellular and inert substrata by EAggEC strains, probably by the cross-talk between probiotic soluble molecules and quorum-sensing mediators of opportunistic strains; so they could be used mainly in the prophyaxis, but even in the treatment of gastro-intestinal chronic disorders, as an alternative or in association with antibiotics.

**P1234 Low virulence associated with fluoroquinolone resistance of uropathogenic Escherichia coli strains**

J. Vranes*, I. Herljecic, M. Senjug, I. Butic, M. Anusic, T. Marijan, V. Leskocar, A. Minaric-Dezpina (Zagreb, HR)

**Objectives:** Escherichia coli is the most frequent cause of urinary tract infections (UTIs), and particular virulence characteristics are associated with ability of strain to cause uncomplicated UTI. The aim of this study was the characterisation of virulence characteristics of E. coli strains isolated from urine of outpatients in Zagreb region, in dependence to fluoroquinolone sensitivity.

**Materials and Methods:** During the five-month study period a total of 2, 451 E. coli strains were isolated from urine of nonhospitalised patients with significant bacteruria. A total of 60 fluoroquinolone-resistant (FR) and a total of 60 fluoroquinolone-sensitive (FS) E. coli strains were randomly collected and characterised. Susceptibility to antibiotics was determined by disk-diffusion and broth microdilution method according to NCCLS (National Committee for Clinical Laboratory Standards). For each strain O-serogroup, adhesion type, production of haemolysin, and the serum sensitivity were examined. Bacterial susceptibility to serum killing was measured by assessing regrowth after incubation in serum, the adhesions were determined by haemagglutination and inhibition of haemagglutination, and serotyping was performed on glass slides.

**Results:** The range of minimal inhibitory concentrations of ciprofloxacin was 8–64 mg/L in the resistant group of strains. All FR strains were co-resistant to amoxycillin, most (39) were co-resistant to trimethoprin/sulafamoxazole, and 20 out of 60 strains were co-resistant to aminoglycosides. Investigated virulence factors were significantly less frequent among resistant isolates. UTI-associated antigens were less prevalent in FR strains than in FS strains with a high frequency of strains with incomplete O-antigen in the resistant group in contrast to the sensitive group of strains (p < 0.01). Haemolysin production and haemagglutination were less prevalent in FR strains than in FS strains as well (p < 0.01), and in 38 (63.3%) and 39 (65%) of resistant strains adhesions and haemolysin were not detected, respectively. The prevalence of serum-resistant strains was significantly higher in FS group of strains, as compared to strains isolated in FR group (p < 0.01), which is in accordance with higher virulence and invasive potential of these strains.

**Conclusion:** These results suggest the association of fluoroquinolone resistance and a decrease in the expression of virulence factors in uropathogenic community-acquired E. coli.
capacity compared with virulence group D. The B1 phylogenetic group represent only 5% and 2.8% of urine and blood isolates, respectively. RAPD M13 profile showed a predominant clone among the E. coli isolates (57.8%, pattern 1) while 42% were distributed among 5 patterns (patterns 2 to 6). Within predominant and persistent (2002–2007) clone 1 the phylogenetic group B2 was prevalent although co-exist with phylogenetic group A. Among the 2 to 6 RAPD patterns all phylogenetic groups were represented. In 91.1% (41/45) of blood isolates were detected the ecpA gene. The four isolates that lacked the EcpA adherence factor were from non frequent clonal profiles. Also all the 26 isolates recovered from 12 patients with UTI and bacteriemia showed the ecpA gene.

Conclusions: Bacteriemia and ITU are usually associated with virulent extra-intestinal phylogenetic group B2 although this study demonstrated high frequency of commensal phylogenetic group A in both urine and blood isolates, suggesting that the gastrointestinal tract can be a relevant primary source for UTI and bacteriemia. Pathogenic E. coli strains may provide to use EcpA to mimic commensal E. coli and confer themselves an ecological advantage for host colonisation and evasion of the immune system.

**P1238 Influence of glycosaminoglycans on Proteus mirabilis-induced urolithiasis**

A. Torzewska*, A. Rozalski (Lodz, PL)

Objectives: Infection stones account for 10–15% of all urinary stones and represent a significant health problem due to their high rate of recurrence and renal tissue damage. These stones occur as a consequence of urinary tract infection by urosepsis-producing bacteria such as Proteus mirabilis. Ammonia, produced by the enzymatic hydrolysis of urea, elevates urine pH causing a supersaturation and a crystallisation of magnesium and calcium ions as the struvite (ammonium magnesium phosphate) and the apatite (calcium phosphate), respectively. Macroorganisms have natural mechanisms of defence including a presence of crystallisation inhibitors in urine. The goal of this study was to establish a role of urinary glycosaminoglycans in struvite urolithiasis as potential inhibitors of this process.

Methods: We used P. mirabilis strain which has been isolated from a human renal stone. The in vitro models were provide to analyse crystallisation and adhesion of crystals to the normal human urothelium (Hu 609, HCV 29 and HRPTEC lines). In these models synthetic urine was also used with or without glycosaminoglycans (GAG−). Crystal analysis with Coulter Counter. Crystal adhesion intensity was analysed using radioactive isotope of calcium.

Results: It was found that in the presence of all tested glycosaminoglycans crystallisation occurred. None of the substances tested had any significant inhibitory effect on crystal growth. In fact, crystallisation was enhanced in the presence of chondroitin sulfate C. It was also found that addition of this glycosaminoglycan caused agglomeration of crystals. Similar influence of these compounds was shown in case of crystals producing Chondroitin sulfate, heparin sulfate and hyaluronic acid). Crystal formation was examined by phase-contrast microscopy and by particle analysis with Coulter Counter. Crystal adhesion intensity was analysed using radioactive isotopes of calcium.

Conclusions: Urinary glycosaminoglycans had no inhibitory effect on infection-induced crystallisation. Our results showed that GAG and GAGA significantly increased crystals binding to epithelium.

**P1239 Heat shock-induced phage shock protein A increases Salmonella typhimurium virulence in BALB/c mice**

N. Ordoszadeh*, A. Shoae Hassani (Tehran, Fars, IR)

Objectives: Salmonella typhimurium is an intracellular pathogen that bring about thousands reported cases of acute gastroenteritis and diarrhoea each year. Although many successful physiological and genetic approaches have been taken to conclude the key virulence determinants encoded by this organism, the totally number of uncharacterised reading frames observed within the S. typhimurium genome suggests that many virulence factors remain to be discovered. This study was conducted to evaluate the role of heat induced phage shock protein A (PspA), in the pathogenicity of S. typhimurium.

Methods: Salmonella typhimurium strain ATCC 14028 (PTCC 1186) was obtained from Persian Type Culture Collection in Tehran, Iran. It was routinely cultured on Trypticase soy agar (TSA, Difco, France). Nine aliquot tubes (10ml) of working cultures (10⁸ CFU/ml) were heat stressed by immersion (3cm above medium level in bottle) at 40°C, 45°C, 50°C, 55°C, 60°C and 70°C temperature controlled water bath gently. The stress proteins that detected on SDS polyacrylamide gel electrophoresis were identified specifically by immunoblotting with polyclonal antibody against PspA. Site directed mutagenesis took place for deletion pspA in control cell line.

Results: Lethal dose of untreated Salmonella typhimurium for 50% of female 6–8 weeks old Balb/c mice inoculated orally was 6 × 10⁵ CFU. At the point of 65°C; stressed bacteria that has produced PspA were more virulent (16 folds greater) to female 6–8 weeks old Balb/c mice.

Conclusion: Correspondency between decrease in LD50 and increasing in PspA during heat stress and lower pathogenicity in non-producing PspA cells that produced by site directed mutagenesis represents PspA as an important virulence factor in heat stressed S. typhimurium.

**P1240 Vibrio species utilise Acanthamoeba castellanii as an environmental host**

H. Abd*, A. Saeed, G. Sandström (Solna/Stockholm, SE)

Objectives: Vibrio is a genus of Gram-negative bacteria found in water and it may be carried by sea living animals. The genus comprises nearly 70 species. Vibrio cholerae O1 and V. cholerae O139 produce cholera toxin and cause cholera. V. cholerae non-O1/O139 strains and other vibrio species such as V. parahaemolyticus, V. vulnificus and V. mimicus can cause gastroenteritis, open wounds infection, and septicemia. The prevalence rate of infections caused by vibrios increases globally. The combination of increased water temperature and salinity may contribute to increased association rates of the bacteria with sea living animals or protozoa. Our recent studies have shown that Vibrio cholerae O1 and O139 have the ability to grow and survive in the aquatic free-living amoeba Acanthamoeba castellanii. The aim of the current study was to highlight interaction of different vibrio clinical isolates with A. castellanii. Vibrio species were isolated from Bangladesh, India and Sweden and they included V. cholerae O1, V. cholerae O139 MO10, V. cholerae O139 SG24, V. mimicus and V. vulnificus.

Methods: Acanthamoeba castellanii and Vibrio strains were alone and co-cultivated for two weeks. Gentamcin assay was used to kill extracellular vibrios as well as to examine ability of amoeba to protect intracellular vibrios from antibiotic killing. Interaction between
In vitro study of dendritic cells maturation induced by Helicobacter pylori strains: evaluation of the inflammatory response and immunological consequences


Objectives: Gastric MALT lymphoma corresponds to B lymphocyte proliferation, which is organised in a lymphoid structure in the gastric mucosa and directly linked to gastric Helicobacter pylori infection. In this context, our aim is to investigate the role of dendritic cells (DC) in response to H. pylori, by studying both cytokine production and microRNAs (miRNAs) expression in well characterised ex vivo activation conditions.

Methods: Human DCs were matured in the presence of IL-4 and GM-CSF, and thereafter co-cultured for 48 h in the presence of H. pylori strains isolated from either low grade gastric MALT lymphoma or duodenal ulcer patients, at a multiplicity of infection of 10. DC surface maturation markers (CD40, CD80, CD83, CD86, CD1a, CD197, HLA-DR) were determined by flow cytometry, and secreted cytokines by ELISA. The ability of -activated DCs to induce allogeneic T lymphocyte proliferation was measured by bromodeoxyuridine incorporation and CD3 expression, both by flow cytometry analysis. DC expression of several miRNAs was determined on total RNAs by quantitative RT-PCR.

Results: Four gastric MALT lymphoma and 4 duodenal ulcer H. pylori strains were tested on DCs. A significant expression was obtained for each maturation markers molecules. All H. pylori strains were able to induce the production of several chemokines such as ENA-78, MIP-1 delta, MCP-1, GRO, GRO-alpha, as well as the cytokines GM-CSF, TNF-α, IL-6, IL-7 and IL-10. A tendency was observed for IL-10 to be more induced by gastric MALT lymphoma strains than by duodenal ulcer strains. High induction of miR-155, miR-146 was also observed whatever the H. pylori strain. Finally, H. pylori-activated DCs were able to induce a significant T lymphocyte proliferation.

Conclusion: Our results show that H. pylori was able to activate DCs ex vivo, thereby promoting T lymphocyte proliferation. We show for the first time that H. pylori is able to strongly induce several miRNAs that have been implied in pathologies such as lymphoma and cancers. This work constitutes the basis of further investigations determining whether H. pylori gastric MALT lymphoma strains orientate the proinflammatory response in a profile favourable to B lymphocyte proliferation.

Relationship between prevalence of peptic ulcer and cagA/iceA genotypes of Helicobacter pylori

I. Alipourfard*, S. Mahmoodi (Tehran, IR)

Objectives: To determine the prevalence of cagA/iceA genotypes Helicobacter pylori (H. pylori) isolated from a group of Iranian patients with gastric complaints, and to find out any significant correlation between these strains and severe gastric clinical outcomes such as peptic ulcer and gastric cancer in Iranian population.

Methods: A total of 918 gastric biopsies from 306 patients who presented with symptoms suggestive of chronic gastritis, peptic ulcer disease, or gastric carcinoma were taken from big and main hospitals in the northern region of Tehran from March 2007 to September 2008. We cultured the samples for H. pylori and then polymerase chain reaction (PCR) was carried out to check for the presence or absence of cagA gene the status of iceA genotypes.

Results: Among the 306 suspected to be infected with H. pylori by means of clinical features and endoscopic findings; 70 patients (23%) were positive using culture technique. Also use of PCR for determine of the cagA gene in these samples showed the relation of the presence of cagA and the development of cases of gastritis and ulcer was statistically significant (p=0.0001). Furthermore, this study revealed that 98.2% of ulcer cases were infected with iceA1 with a statistically significant correlation (p=0.0001), while 92.5% of gastritis and 88.1% of normal were infected with iceA2 (p=0.0001). Moreover cagA+/iceA1 combined genotypes was statistically correlated with peptic ulcer (100%) but not cagA−/iceA1 (0%; p=0.0001).

Conclusion: Certain H. pylori genotypes were more virulent than others. Multiple clinical implications based on these finding might be studied further.
Circulating plasmablasts with reactivity against individuals' own intestinal microbiota in patients with appendicitis

S. Pakkane*, N. Pulkola, N. Rossi, R. Puohintom, J. Kantele, A. Kantele (Helsinki, Jyväskylä, Turku, FI)

Objectives: The microbes found in inflamed appendix belong to the normal microflora of the intestine, but they appear to become causative microbes in appendicitis. We wanted to see what kind of an immune response is mounted against these microbes. We looked for microbe-specific circulating plasmablasts in patients with appendicitis.

Methods: 13 patients with acute appendicitis were investigated. Microbes were cultured from each patient's own inflamed appendix samples. Peripheral blood mononuclear cells (PBMC) were isolated and microbe-specific ASC were enumerated using ELISpot, where cells are secreting antibodies in microtiter plate wells coated with bacteria isolated from each patient's own inflamed appendix after appendicectomy.

Results: In patients with appendicitis, 1−4 microbe strains were isolated from the inflamed appendix. Microbe-specific antibody-secreting cells appeared in the circulation in all patients with appendicitis. In most cases the response was dominated by IgA-ASC. In microbiocidal cases, the magnitude of the response varied between the pathogens.

Conclusions: Microbe-specific antibody-secreting cells appear in the circulation in patients with appendicitis. This immune response is dominated by the mucosal Ig-isotype, IgA. Variations in the magnitude of the response between pathogens may reflect different clinical significance of the microbes.

Cloning and characterisation of EngA, a GTP-binding protein from Mycobacterium tuberculosis H37Rv

M. Laxman Singh*, R. Laxman Singh (New Delhi, IN)

Guanine nucleotides are critical elements and key signaling molecules. Many members G protein family bind and hydrolyze such nucleotides, particularly GTP, and regulate the intracellular level of GTP and GDP. The structure and sequence motifs of the GTPase are highly conserved in all organisms, ranging from prokaryotes to eukaryotes. Comparative genomic approach was used to predict homologs of GTPase in Mycobacterium genome. Guanine acid sequence alignment of EngA of M. tuberculosis H37Rv with three other homologous bacterial proteins have shown that EngA of M. tuberculosis H37Rv has significant homology with EngA of other bacteria and DXG motif, which is a characteristic feature of all known GTPases. EngA of M. tuberculosis H37Rv was cloned and expressed in E. coli. Purified protein showed GTP binding and hydrolysing activity. This study, confirmed that newly cloned and expressed homologs not only had G protein functionality but that known key residues in well-established G proteins were also key residues in the homologs, thus indicating that these homologues are indeed G proteins as well. Studies are in progress to understand the physiological significance of these proteins in M. tuberculosis H37Rv.

Gastrointestinal infections

One-year perspective study on prevalence and characterisation of diarrhoeagenic Escherichia coli isolated from children, beef and cattle in Tehran, Iran


Objective: Several routes exist for human infection with Diarrhoegenic Escherichia coli (DEC) and Meat and it's products remains a main sources. The purpose of this study was to investigate the prevalence of five important categories of DEC in Cattle faeces, Beef and children with acute diarrhoea in Tehran, Iran.

Methods: From December 2007 to 2008, four hundred and sixty six samples, including 222 cattle faeces collected from healthy cattle (1–3, 4–6 and >6 months) in farm, 104 beef samples at the slaughterhouses and 140 stool specimens from children (Age categories: 0–6, 7–12, 13–24 and 25–60 months) with diarrhoea, who referred to Tehran children's hospital were tested. All samples were investigated and identified for DEC and their virulence genes (stx1, stx2, eae, Lt, St, iai, ipaH, bfp, tcc, O157, H7, SOD and a-hly genes) by Conventional and Molecular techniques.

Result: A total of 270 (58%) of 466, one hundred and sixty seven (75.2%) of 222 faecal samples which, collected from healthy cattle in farm, 81 (77.8%) of 104 beef samples at the slaughterhouses and 13 (9.2%) of 140 stool specimens from children with diarrhoea were positive for the stx genes (STE). Of the 270 STEC isolates, 15% harboured the stx1 gene, 23% harboured the stx2 gene and 62% carried both stx1 and stx2 genes. The eae gene, was significantly associated with the stx1 and stx2 genes in Meat samples (38%) and Cattle faeces (51%). 98 of 167 STEC positive, which were detected from animal faeces were carried eae gene (58.7%). Enteropathogenic E. coli (EPEC) strain was isolated from 23% of bovine faeces, 19% of beef samples, and 14.1% of stool specimens of children. A PCR indicated that 6.8%, 5%, 14%, 10.7% and 13.6% strains carried the a-hly, hlst, ipaH, iai, and Pcvd432 genes in stool specimens of children respectively. SOD gene was positive in 18.6%, 17.3% and 37.43% of STEC strains, wch are detected from Children, Meat and Cattle faeces respectively. Bloody diarrhea was observed in 30.7% of children's stool.

Conclusion: The Result showed the most common DEC in children was Enteropathogenic E. coli (14.1%) and STEC was isolated from large number of the Meat samples (77.8%). Since the STEC are spread only via faecal excretion, at present it is most important to reduce the faecal shedding and to avoid faecal contamination of food of animal origin. In detail prophylactic hygienic measures concerning the Farm management, the Feeding hygiene, the Meat hygiene as well as Food hygiene are need.
**Gastrointestinal infections**

**P1249** Analysis of the gut microbiota of irritable bowel syndrome patients and healthy volunteers using selective media and Real-Time PCR

V. Vankercenhoven*, C. Lammens, S. Chapelle, A. Vanderstraeten, N. Hens, G. Molenberghs, H. Goossens (Witrijk, Hasselt, BE)

**Objectives:** It has been hypothesized that disturbances in the intestinal flora could be a factor in the onset and persistence of IBS complaints. The aim of the study was to analyse the faecal flora of IBS patients and healthy volunteers and to investigate differences in its composition.

**Methods:** Faecal samples were collected from IBS patients (n = 55) fulfilling the Rome II criteria and age and gender-matched healthy volunteers (n = 30). Microbial populations (Bacteroides, anaerobes, bifidobacteria, coliforms, clostridia, and lactobacilli) were enumerated using selective and differential media. Total bacterial counts were performed using DAPI for 28 volunteers and 26 IBS patients to date. Bacterial DNA was analysed by quantitative RT-PCR for the detection of bifidobacteria, Clostridium coccoides and Clostridium leptum of 22 IBS patients and all healthy volunteers. Statistical analysis was performed using an unpaired T-test in case of normal distribution or Kruskal-Wallis. P < 0.05 was considered statistically significant.

**Results:** Large inter-individual variations in the composition of the faecal flora were observed in both groups. Total bacterial counts were >1010 cfu/g faeces in most samples. The largest populations were composed of strict anaerobes, bifidobacteria and Bacteroides, followed by clostridia. Lactobacilli were found at an intermediate level. Total bacterial counts were significantly higher for IBS patients than for healthy volunteers (P = 0.002). Also, significantly higher amounts of lactobacilli were observed in IBS patients than in healthy volunteers (P = 0.03). In contrast, healthy volunteers had significantly higher amounts of anaerobes (P = 0.04) and bifidobacteria (P < 0.001) than IBS patients.

Using RT-PCR, no statistically significant differences were seen between healthy volunteers and IBS patients, although the latter showed a trend for lower amounts of bifidobacteria than healthy volunteers (P = 0.05). This finding could be further confirmed by analysing a larger number of IBS patients.

**Conclusions:** The dominant microbial populations in the faeces of both healthy volunteers and IBS patients were similar. Both quantitative plating and Real-Time PCR results indicate the presence of quantitative alterations in the gut microbiota of IBS patients. The combination of both quantitative plating and quantitative Real-Time PCR provides a targeted approach to enumerate and identify bacterial populations and enables us a better understanding of the GI tract flora.

**P1250** Irritable bowel syndrome among a cohort of European travellers to low income destinations

R. Pitzurra, A. Tschopp, C. Hutz*, R. Steffen, M. Mitsch (Zurich, CH)

**Objectives:** To determine the risk of irritable bowel syndrome (IBS) among European travelers to low income countries and to investigate classic traveller's diarrhoea (TD) and other potential risk factors.

**Methods:** Adult travellers consulting our travel clinic for pre-travel health advice were invited to participate in a cohort study before leaving to a high risk TD destination for a maximum of 8 weeks between July 2006 and January 2008. Participants were investigated about demographics, travel and health characteristics by means of questionnaires pre-travel (Q1), immediate post-travel (Q2) and 6-months post-travel (Q3). Exclusion criteria included pre-existing functional gastrointestinal disorders and antibiotic prophylaxis. IBS and related symptoms were assessed pre-travel and 6-months post-travel according to Rome III criteria.

**Results:** Among 3,100 travellers enrolled (Q1), 2,800 (90.3%) concluded Q2 and 2,440 (78.7%) were eligible for the final analysis. Classic TD was reported by 837 returning travellers (34.3%). Symptoms compatible with IBS were recorded in Q3 in 31 (1.3%) travellers and a 2-weeks-of-stay incidence of 0.9% was estimated. In a preliminary multivariate analysis classic TD was an independent risk factor of IBS (RR 4.8, 95%CI 2.2–10.4). Additionally, age, newcomers to tropics and sub-tropics and reported consumption of potentially contaminated food and beverages significantly increased the risk of IBS. No significant difference was found for gender, travel duration, travel destination and education.

**Conclusions:** The incidence of travel-related IBS in Europeans is lower than the one in other populations.

**P1251** Randomised health-point surveillance of human gastro-intestinal parasites among patients attending a teaching hospital in Ishaka, Uganda

E. Agwa*, G. Tanavyen (Ishaka, UG)

**Background/Objective:** The upsurge of: poverty; shortage of clean drinking water; poor nutrition, health education, health-facilities, personal and environmental hygiene in sub-Saharan Africa has raised infection due to human gastro-intestinal parasite to a public health dimension. This study was designed to determine the prevalence of intestinal parasites among patients attending KIU-TH Ishaka, Bushenyi, Uganda.

**Materials and Methods:** Standard parasitological methods were used under aseptic conditions to screen stool samples for intestinal parasites. Seventy six (26 males and 50 female) out patients diagnosed with lower abdominal pain and gastro-intestinal discomfort at KIU-TH were recruited for this investigation. Patients on anti parasitic agents were excluded. Chi square was used to test for statistical significance of result obtained. (p = 0.05).

**Results:** The overall prevalence of parasites was 52 (68.4%). The most prevalent parasites were Entamoeba histolytica 17 (22.4%) followed by Entamoeba coli 11 (14.5%) and Ascaris lumbricoides 7 (9.2%). Giardia lamblia 2 (2.6%) and Trichomonas hominis 2 (2.6%) were equally prevalent while the observed 7.9% co-infection of Ascaris spp and E. histolytica was the highest coinfection rate followed by 3.9% co-infection of Ascaris lumbricoides and Giardia lamblia, 2.6% Trichomonas hominis and Ascaris lumbricoides. There was similar male/female prevalence ratio (69.2%; 68.0%) of intestinal parasites. Most patients above 10 years were peasant farmers. The highest age specific prevalence (89.5%) was observed among age group 21–30 years. This was followed by 81.8% of patients, 41–50 years of age; 80.0% in age group 11–20 years; 61.5% in age group 31–40 years; 60.0% in age group 51–60 years and 50.0% in age group >60 years. There were statistical significant differences (p < 0.05) when occupation, sex and age groups were tested depicting their role in the epidemiology of parasitic infections in the studied population.

**Conclusion:** Intestinal parasites were highly prevalent (68.4%) and Ascaris spp and Entamoeba histolytica occurring both as single and mixed infection are the most predominant parasites causing lower abdominal pain and intestinal discomfort in Bushenyi. Parasitic prevalence were significantly (p < 0.05) dependent on age, occupation and sex. More studies are needed to determine prevalence in different age and occupational settings. Intervention strategies are paramount in reducing infection to barest minimum.

**P1252** The cost-effectiveness of hospital closure to control norovirus outbreaks

Z. Sadique*, B. Logman, B. Cooper, J. Edmunds (London, UK)

**Objective:** To estimate the cost and cost effectiveness of hospital ward closure policy to control norovirus outbreak in the UK hospitals.

**Methods:** Intensive gastroenteritis surveillance data has been used to investigate the transmission of norovirus within the hospital. On the basis of the parameters from transmission model an epidemiological model was constructed to find out the outbreak of norovirus in a hospital environment. Economic analysis was based on the outcome of the epidemiological model. This analysis measured the costs and benefits of hospital closure, where the policy intervention was to close a ward.
when there is an outbreak. The closure policy was varied between 1, 3, and 5 days since first notification of outbreak.

Results: Uncontrolled norovirus outbreaks are estimated to cost an acute hospital around £0.12 million yearly. The cost of intervention, i.e., closing wards to new admissions varies between £0.5 million to £0.9 million depending on the effectiveness of closure in controlling within hospital transmission of infection, and also when ward closure is put into place.

Conclusion: Ward closure helps to avoid significant amount of clinical cases. Closure of wards reduces cases by around 50%, which has important implication for both hospital and patient in terms of saving resources.

**Real-time comparison of Listeria monocytogenes PFGE profiles of human and food isolates for enhanced epidemiological investigation of listeriosis**

S. Lukinmaa*, L. Rantala, M. Kausi, T. Johansson, A. Sitionen (Helsinki, FI)

Objectives: In Finland, the number of Listeria monocytogenes cases has varied from 20 to 50 with an average incidence of 7 cases per million inhabitants annually. In the Enteric Bacteria Laboratory of the former National Public Health Institute (KTL), now the National Institute for Health and Welfare (THL), all human L. monocytogenes isolates have been PFGE-(pulsed-field gel electrophoresis) typed since 1990 and small infection clusters are detected every year. However, in most of the cases the source of infection has remained unclear for several reasons and a press release was usually given to inform consumers to avoid risk products. In Finland, certain PFGE types have been strongly connected with vacuum-packed fishery products and, therefore, intensified hygiene measures have been implemented in fishery establishments. As well, in early 2008, the Finnish Food Safety Authority Evira started a one year national monitoring programme focusing on vacuum-packed fishery products at the retail level. Furthermore, to enhance the detection of infection clusters and rapid tracing of the source of infection, THL and Evira started a real-time comparison of PFGE profiles of L. monocytogenes isolates from humans and foodstuffs.

Methods: All PFGE profiles of human isolates and those associated with Evira’s national survey as well as those from official control samples sent by the regional food control laboratories to Evira are included in the comparison study. The Ascl-PFGE typing is carried out using the PulseNet protocol.

Results: During January–September 2008 among 32 human and 331 food isolates (from 166 samples), 12 and 42 different PFGE types were found, respectively. Seven of the human PFGE types were simultaneously detected in food, mainly in fishery products. PFGE type 7, which caused an infection cluster of five cases in August–September, was not isolated from food during this study period. Of the human PFGE types found, four have not been discovered in food.

Conclusion: The laboratory-based surveillance and comparison of the PFGE profiles will be continued to gain more knowledge of the real-time biodiversity of infective L. monocytogenes population in foodstuffs. The data produced will enhance the epidemiological investigations to find out the sources of infection clusters. Supposedly, fish is one of the main sources, however, the epidemiological links are missing. Furthermore, our data also showed that certain types have only been detected in human samples.

**Gene mutations of 23S rRNA and rdxA deletion associated with clarithromycin & metronidazole resistance in Helicobacter pylori strains isolated from UAE patients**

M. Alfaresi*, W. Altyayari, A. Elkouch (Abudhabi, Alain, AE)

Objective: To determine the prevalence of antibiotic resistance genes (mutation in 23S rRNA gene in clarithromycin, and deletion in rdxA gene in metronidazole) among H. pylori strains isolated from U.A.E patients by using molecular methods PCR and Sequencing.

Methods: DNA was extracted from antral gastric biopsy samples from 90 dyspeptic patients. Primary screening for H. pylori were done by Clo test at endoscopy department in Zayed Military Hospital (ZMH). All samples were confirmed for positive H. pylori by PCR. Mutations of the corresponding gene were studied by PCR and sequencing technique. DNA sequence editing and analysis were performed by ClustalX, version 2.

Results: Out of 90 biopsy samples 26 were positive for H. pylori by PCR and 22 by Clo test. Resistance to clarithromycin and metronidazole was detected in 9 (34.6%) and 3 (11.5%) strains, respectively. Of the clarithromycin resistant strains, 22.22% had the A2142G mutation in the 23S rRNA gene, 55.56% A2143G, and 11.11% A2143C and 11.11% of highly changed in sequence. Of the metronidazole resistant strains, deletion in rdxA gene was detected in 3 strains which were negative for Clo test.

Conclusion: A significant proportion of gastric mucosal biopsies obtained in the UAE is positive for Genes associated with Clarithromycin and Metronidazole resistance(mainly in Clarithromycin). A2143G remains the most prevalent point mutation involved, thus suggesting that new therapeutic strategies are needed.

**The prevalence of diseases associated with Helicobacter pylori in St. Petersburg, Russia at present**

R. Ferman, A. Zhebrun, O. Balabash, A. Scarvul* (St. Petersburg, RU)

Helicobacter pylori infection is a matter of pressing concern of the Russian Public Health. In 2008 we conducted serological tests of 463 individuals including 129 children and adolescents aged 0–19 and 334 adults aged 20–82. The screening of patients with chronic gastritis, chronic gastroduodenitis, peptic ulcer, malignant neoplasms in gastrointestinal track and individuals without clinical manifestations of Helicobacter pylori infection was carried out.

Methods: IgG screening for H. pylori and Cag A H. pylori antibodies was performed using ELISA method.

**Results:** The results of the screening showed that 48 examined children and adolescents (37.21%) and 164 examined adults (49.10%) possessed antibodies for toxin-associated protein Cag A H. pylori. Antibodies for bacterial H. pylori antigen were discovered in the screening of 45.74% of the examined children and adolescents and of 66.51% of adult patients. Thus, up to 20% of non-toxigenic strains of H. pylori are circulating among the population and play a role in the development of diseases. We can conclude that the lowest rate of infected individuals (25%) is defined in the age group 0–5 years old, whereas for 6–12 years old group this rate is 35%, and the adolescents’ group aged 13–19 showed 50% rate of the infection. The infection rate among adults is 20–29 y.o. group and in 30–39 y.o. group, which is correspondingly equal to 52.72% and 54.83%. The number of H. pylori infected individuals is decreasing to some extent with age – the infection rate goes down to 49.08% for individuals over 50 years old. Therefore, adolescents and young people can be considered to be the main risk group for the prevalence of H. pylori infection and diseases associated with it, i.e. the inverted character of age-dependent dynamics of H. pylori infection was identified.

Conclusions: Contemporary epidemic situation regarding H. pylori infection can be characterised by high circulation rate of this pathogen microorganism and by no evidence of the infection rate decrease among young generations, in other words, the conducted study did not show any signs of so-called “cohort effect” that have been observed in some countries in Western Europe and North America. The findings correlate with an increase of the diseases associated with H. pylori among children and adolescents. Further consideration of these peculiarities of the contemporary epidemic situation is necessary.
**P1256** Resistance to antibiotics of *Helicobacter pylori* strains from patients after treatment failure

M.T. Massellino*, M. Murgani, M.P. Hassenem, A. Olica (Rome, IT)

Objectives: Aim of our work was to evaluate the "in vitro" resistance of *Helicobacter pylori* (Hp) in patients who underwent from 2 to 9 empiric therapy cycles after a wash-out period of at least 4 weeks with no antibiotic and anti-reflux therapy.

Methods: The population consisted of 25 out-patients, in whom at least two eradication regimens for Hp infection had failed. Due to the different distribution of the microorganism in the stomach, biopsy specimens were drawn from different sites (antrum, fundus, corpus) and inoculated on Pylori Selective agar. The bacteria identification was performed by oxidase, urease and catalase tests. Antibiotic susceptibility was tested by Kirby-Bauer and E-test for metronidazole (MZ), levofloxacin (LEV), tetracycline (TE), clarithromycin (CLA) and amoxicillin (AMX).

Results: From a total of 75 specimens (3 for each patient), 32 strains of Hp were isolated in 14 subjects out of 25 (56%) (3 antritis and 11 gastritis). Hp strains from the 3 gastric regions resulted to be different in 2 subjects. No discrepancies between the two methods used were observed except for 15.62% (5 strains) but with a higher number of intermediate (9.38%). Resistance to the same antibiotic of the 11 patients with pangastritis, 81.8% (9/11) showed a concomitant for MZ concerning 2 strains that showed resistance only by E-test. Out of the 11 patients with gastritis, 81.8% (9/11) showed a concomitant colonisation of the 3 gastric regions. The resistance to the same antibiotic from each gastric region resulted to be different in 2 subjects.

Conclusion: The presence of mutant resistant bacteria can be due to the prolonged and continuous exposure to antimicrobial agents during the infection with consequent treatment failure or to the use of excessively low doses of antibiotics during the initial treatment. K-B and E-test provide comparable results for Hp when testing for the antibiotics tested with reduced reliability for MZ. In fact the E-test may over-estimate MZ-resistance because of the presence of intermediate MIC levels. Dual resistance to both MZ and LEV was found in 18.76% (6 strains); resistance to TE corresponded to 15.62% (5 strains) but with a higher number of intermediate (9.38%). No discrepancies between the two methods were observed except for MZ concerning 2 strains that showed resistance only by E-test. Out of the 11 patients with gastritis, 81.8% (9/11) showed a concomitant colonisation of the 3 gastric regions. The resistance to the same antibiotic from each gastric region resulted to be different in 2 subjects. No discrepancies between the two methods used were observed except for 15.62% (5 strains) but with a higher number of intermediate (9.38%). Resistance to the same antibiotic of the 11 patients with pangastritis, 81.8% (9/11) showed a concomitant for MZ concerning 2 strains that showed resistance only by E-test. Out of the 11 patients with gastritis, 81.8% (9/11) showed a concomitant colonisation of the 3 gastric regions. The resistance to the same antibiotic from each gastric region resulted to be different in 2 subjects. No discrepancies between the two methods used were observed except for 15.62% (5 strains) but with a higher number of intermediate (9.38%). Resistance to the same antibiotic of the 11 patients with pangastritis, 81.8% (9/11) showed a concomitant for MZ concerning 2 strains that showed resistance only by E-test. Out of the 11 patients with gastritis, 81.8% (9/11) showed a concomitant colonisation of the 3 gastric regions. The resistance to the same antibiotic from each gastric region resulted to be different in 2 subjects.

**Table 1. Distribution of MICs (mcg/ml) and sensitivity, intermediate and resistance values for 32 *Helicobacter pylori* isolates from 25 patients under consideration.**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mcg/ml)</th>
<th>Sensitivity</th>
<th>Intermediate</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ</td>
<td>&lt;0.25</td>
<td>≥90%</td>
<td>≤10%</td>
<td>≤10%</td>
</tr>
<tr>
<td>CLA</td>
<td>&lt;0.03</td>
<td>≥97%</td>
<td>≤3%</td>
<td>≤3%</td>
</tr>
<tr>
<td>TE</td>
<td>&lt;0.5</td>
<td>≥95%</td>
<td>≤5%</td>
<td>≤5%</td>
</tr>
<tr>
<td>LEV</td>
<td>&lt;0.03</td>
<td>≥95%</td>
<td>≤5%</td>
<td>≤5%</td>
</tr>
<tr>
<td>AMX</td>
<td>&lt;0.03</td>
<td>≥95%</td>
<td>≤5%</td>
<td>≤5%</td>
</tr>
</tbody>
</table>

Legend: MZ = Metronidazole, CLA = Clarithromycin, AMX = Amoxicillin.

**P1257** Different strains of pathogenic *Versinia enterocolitica* distributed among wild boars and domestic pigs in Switzerland

M. Fredriksson-Thoma*, S. Wachek, R. Bonke, M. Koenig, A. Stolle, R. Stephan (Oberschleissheim, DE; Genesa, Zurich, CH)

Objectives: Domestic pigs are an important reservoir of human pathogenic *Versinia enterocolitica* in many countries including Switzerland. In the past years, wild boar population has increased considerably in Europe and at the same time outdoor rearing of domestic pigs has become more popular. High density of wild boars and increasing number of outdoor pigs increase the risk of transmission of zoonoses between wild boars and domestic pigs. The aim of this study was to compare pathogenic *Y. enterocolitica* strains isolated from wild boars and domestic pigs in Switzerland.

Methods: Fourteen pathogenic *Y. enterocolitica* strains from wild boars were compared with 78 strains from domestic pigs. The wild boar strains were isolated from tonsils of 14 out of 153 (9%) animals shot in Switzerland between October 2007 and March 2008. The domestic pig strains were isolated from tonsils of 72 out of 212 (34%) pigs at slaughter in Switzerland during February and March 2006. The strains were bio- and serotyped. The presence of several virulence genes (aui, yst, hreP, vuR and yadA) were studied by PCR and the genotypes was studied by PFGE using NotI, Apal and Xhol enzymes. Additionally, antimicrobial resistance analysis against 16 antimicrobial agents was performed with disc-diffusion test.

Results: No bioserotype dominated among wild boar strains but bioserotype 4/O:3 dominated (91%) among domestic pig strains. The wild boar strains belonging to human pathogenic bioserotypes carried all virulence genes studied. Some (25%) of the domestic pig strains belonging to bioserotype 4/O:3 were negative for plasmid-borne virulence genes. All wild boar strains were resistant to amoxicillin/clavulanic acid but all domestic pig strains of bioserotypes 4/O:3 and 2/O:9 were sensitive. All wild boar strains were sensitive to sulphametoxazol, trimethoprim and trimethoprim/sulphamethoxazole but some (4) of the domestic pig strains showed resistance. All genotypes of wild boar strains differed from domestic pig strains. Especially strains belonging to bioserotype 4/O:3 were clearly different with all three enzymes.

Conclusions: Distribution of human pathogenic bioserotypes and resistance to amoxicillin/clavulanic acid among wild boar strains was different from domestic pig strains. Furthermore, all genotypes of wild boar strains differed from domestic pig strains, which indicates that wild boars and domestic pigs so far are reservoirs for different strains of human pathogenic *Y. enterocolitica*.

**P1258** Is *Clostridium difficile* 027 more virulent than other ribotypes? Report of the first outbreak of *Clostridium difficile* ribotype 027 in East of England: correlation of outbreak and non-outbreak ribotypes with severity of disease

N. Elumogo*, T. Hegarty, G. Vautier, T. Chinniah, L. Hawthin, L. Warner, B. Rechel (Gorleston, Beccles, UK)

Objectives: James Paget University Hospital (JPUH) is a 550 bedded medium-sized acute General Hospital in the coastal town of Great Yarmouth, East of England serving a population of approximately 225,000 people which increases significantly during the holiday seasons. We report an outbreak of *Clostridium difficile* 027 Infection, the first in the East of England. The outbreak occurred between December 2006 and 14th April 2007 and affected 221 patients. We correlate clinical severity of disease and outcome with ribotypes.

Method: The outbreak was recognised due to rising numbers of cases and rapidly progressive clinical disease. Fifty six patients were diagnosed in the community and 165 patients were in or associated with the JPUH. Control measures were formulated using the *Clostridium difficile* associated disease (CDAD) acronym by the first author:

- Contact precautions and isolation in a dedicated ward
- Disinfection and cleaning with hypochlorite
- Antibiotic Prudence (restriction of quinolones, cephalosporins and clindamyacin)
- Decontamination of hands with soap & water

Results: Pre-outbreak typing (Jan and Feb 2006) showed 80% ribotype 01B, 10% ribotype 106 and 10% ribotype 72, none was ribotype 027. Outbreak strains (Jan 2006 to April 2006) showed 85% ribotype 027, 15% were made up of 6 different ribotypes. Complications and mortality among cases of ribotype 027 were significantly more than for non 027 strains. There was increased admission to in Intensive care unit, 7 colectomies, 35% all cause mortality and 10% attributable mortality. Post outbreak typing showed greater diversity (11 different types) confirming cases were no longer linked. Although ribotype 027 persisted at 40% clinical severity and mortality had reduced substantially due to heightened awareness and prompt treatment.

**Table 1. Distribution of MICs (mcg/ml) and sensitivity, intermediate and resistance values for 32 *Helicobacter pylori* isolates from 25 patients under consideration.**
Conclusion: An outbreak of *Clostridium difficile* ribotype 027 occurred in JPUH. Ribotyping undertaken before, during and after the outbreak provided robust evidence of the exact time of introduction of ribotype 027 in to the hospital (Summer/Autumn 2006). The availability of ribotype data allowed us to correlate clinical severity, complications and outcome with ribotypes. To our knowledge this is the first report from the UK that is able to document this correlation. We conclude that prompt diagnosis, treatment and community follow up are crucial in reducing complications and mortality from *C. difficile* irrespective of the ribotype.

Figure: *Clostridium difficile* episodes – December 2006 to December 2007.

**P1259 In vitro and in vivo comparison of Clostridium difficile PCR ribotype 027 and non 027 strains**
K. Truusala*, I. Smidt, P. Hütt, S. Köljalg, R.H. Mikelsaar, P. Naaber, E. Seep, J. Stepetcua, M. Mikelsaar (Tartu, EE)

**Objectives:** To compare *C. difficile* strains of PCR ribotype 027 and *VPI 10463* in vitro and their ability to cause infection in Syrian golden hamster model.

**Methods:** *C. difficile* reference strain *VPI 10463* (ATCC 43255) and clinical strain M 13042 (PCR ribotype 027) were tested for toxicity genes using PCR. Antagonistic activity of 14 different human lactobacilli was assessed in vitro against the two *C. difficile* strains by streak line method and spot agar test. In hamster model ampicillin (60 mg/kg) administration (on day −1) was followed by inoculation with 106 vegetative cells of *C. difficile* (on day 0). Five hamsters were infected with *VPI 10463* and 5 with *C. difficile* 027 strains. Quantitative cultures from heart blood, liver, spleen, small and large intestines were performed at autopsy and histological samples were taken on day 5.

**Results:** Both *C. difficile* strains harboured full set of toxin genes (A, B, C, D, E, cdd-3, cdu-2). The inhibition zone of lactobacilli against *VPI 10463* was 0–4 (median 0.65 mm) and against *C. difficile* 027 strain 0–5 (median 2.5) mm by streak line method. Similarly, in spot agar test *C. difficile* 027 demonstrated higher sensitivity (median score 3 vs. 2). All hamsters from *VPI 10463* group died within 48 hours, while in *C. difficile* 027 group two animals died within 48 h, two within 96 h and one survived until day 5 (mortality 100% vs. 80%, respectively). The caecal median counts of *C. difficile*, anaerobes and lactobacilli did not differ significantly in *VPI 10463* and *C. difficile* 027 groups (*C. difficile* 7.0 log CFU/g vs. 6.0 log CFU/g; anaerobes 7.5 log CFU/g vs. 8.9 log CFU/g; lactobacilli 7.8 log CFU/g vs. 7.0 log CFU/g, respectively). *C. difficile* was not detected in the small intestine of the survived hamster, in caecum the counts of *C. difficile* and lactobacilli were 5 log CFU/g and the count of anaerobes was 9.3 log CFU/g. In caecum no relationship was found between the counts of *C. difficile*, lactobacilli and anaerobes. The main finding of histological evaluation was hyperaemia and haemorrhages in different organs.

**Conclusion:** In Syrian golden hamster model the reference *VPI* strain caused more rapid lethal infection as compared to the *C. difficile* 027 strain. This could be associated with higher sensitivity of *C. difficile* 027 strain to protective lactobacilli.

**P1260 Germinate to exterminate: Susceptibility of germinating spores of Clostridium difficile ribotype 027 to desiccation and aerobic conditions**
C.J. Lowden*, L.J. Wheelon, P.A. Lambert, D.L. Rathbone, T. Worthington (Birmingham, UK)

**Objectives:** The effect of desiccation and aerobic conditions on dormant and germinating spores of *C. difficile* ribotype 027 in the presence and absence of a soil load was investigated.

**Methods:** A controlled carrier test system was designed comprising inoculation of stainless steel disks (1 cm²) with a spore suspension of *C. difficile* ribotype 027 followed immediately by the addition of 2% w/v sodium taurocholate in thioglycollate medium. Control disks contained spore suspensions only, without the addition of germinant. Spore suspensions were allowed to dry on carrier disks in aerobic conditions over the course of the experiment, (to simulate a clinical setting), and were compared to spore suspensions which were kept hydrated. To simulate a faecally contaminated environment, the experiment was repeated in the presence of a soil load comprising 5% w/v tryptone, 5% w/v bovine serum albumin, 0.4% w/v mucin in sterile 0.9% w/v saline.

**Results:** Germinating spores showed a 3 log (99.9%) reduction in viability within 5 hours and up to a 4 log (99.99%) reduction over 24 hours when allowed to dry in aerobic conditions in both the presence and absence of a soil load. Germinating spores which were kept hydrated showed less than a 1 log reduction after 5 hours in aerobic conditions in the presence and absence of a soil load, and up to a 3 log reduction after 24 hours in the presence and absence of a soil load. There was no log reduction in viability of dormant *C. difficile* spores after 24 hours exposure to room air in either hydrated or dried conditions, with or without soil loading.

**Conclusion:** Spores of *C. difficile* 027 exposed to an appropriate germination solution become rapidly susceptible to desiccation and aerobic conditions in the presence and absence of a soil load. Dormant spores remain resistant to desiccation and aerobic conditions. Use of a germination solution in the clinical setting may provide a novel strategy ("germinate to exterminate"), in addition to current infection control procedures, for controlling *C. difficile*. Further studies are warranted.

**P1261 Toxigenicity and resistance to antibiotics of Clostridium difficile strains isolated from patients of a tertiary hospital in Greece during a six-year period**
M. Orfanidou*, E. Vagiakou, E. Petrou, K. Savvidou, P. Karabogia-Karafillidis, H. Malimou-Lada (Athens, GR)

**Objectives:** To investigate the toxigenicity and the resistance to antibiotics of *C. difficile* strains isolated from hospitalised patients suffering from *C. difficile* associated diarrhoea, in a tertiary hospital in Athens, Greece, during a six year period (3/02–3/08)

**Methods:** During the study period 4363 diarrhoeic stool samples were examined for *C. difficile* using cycloserine-cefoxitin-fructose agar with 5% egg yolk and cycloserine-cefoxitin blood agar (BD). The strains were identified by rapid ANA II (Remel, Lenexa) and latex test (Culturette, BD). Toxin A was detected by an ELISA (Vidas, bioMerieux) and a chromatographic assay (ColorPac, BD). Both toxins A&B were detected by an ELIA (Premier Toxins A&B, Meridian) and a chromatographic assay (ImmunoCard Toxins A&B, Meridian). Antibiotic susceptibility testing was performed by E-test (AB Biodisk, Solna) according to the manufacturer's recommendations and CLSI's breakpoints.

**Results:** *C. difficile* strains were isolated in 362/4363 (8.3%) stool specimens. Strains A+B were detected in 264/362 (72.9%), A+B+ in 50/362 (13.8%) and strains A-B were found to be 48/362 (13.3%). The resistance rate of the isolated *C. difficile* strains to penicillin was 81.2%, clindamycin 58.4%, tetracycline 32.4%, while no resistance was observed to metronidazole, vancomycin and piperacillin/tazobactam, although one strain presented to vancomycin a high level MIC 4 μg/ml. Especially for meropenem the resistance rate was 7.7%, while 258 strains were tested also to ertapenem and were found to be resistant 22.3% of...
them. The resistance rate of moxifloxacin and erythromycin for 268 Cd strains was found to be 35.4% and 43.4%, respectively. Linezolid was tested in 291 strains and all were found susceptible, while 14 strains were also found fully susceptible to daptomycin and tigecycline. All strains A-B+ producers were found resistant to penicillin, clindamycin and erythromycin

Conclusions: Cd strains A-B+ were the most prevalent but there was an increase of the A-B+ strains, as well as of non toxigenic strains. The resistance rate to penicillin and carbapenems was high, while no resistance was observed to metronidazole, vancomycin and linezolid.

P1262 Resistance of Clostridium difficile to antibiotics in a teaching hospital in Madrid: current situation

C. Bernaola, J. Perez*, E. Amor, C. Betriu, J. Picazo (Madrid, ES)

Objectives: Metronidazole and vancomycin are the drugs of choice for treatment of Clostridium difficile (CD)-associated disease. In Spain, there have been reports of resistance of CD to metronidazole and intermediate resistance to vancomycin. This study aims to determine the frequency of these resistant isolates in our hospital.

Methods: We studied the sensitivity of 100 non duplicate toxigenic strains of CD obtained between October 2007 and October 2008 against 14 antimicrobial drugs using the microdilution method according to CSLI guidelines. We compared the results with those from a similar study carried out 10 years ago. We also studied sensitivity to metronidazole, vancomycin, daptomycin, and tigecycline using the E-test.

Results: We found 2 isolates with an MIC of 16 mg/L (2%) by both methods. All the isolates were uniformly sensitive to vancomycin, amoxicillin-clavulanic acid, piperacillin, and piperacillin-tazobactam. Resistance to amoxicillin, imipenem, tetracycline, and clindamycin was greater than in the previous study. Moxifloxacin showed 40% resistance. Both tigecycline and daptomycin showed good activity, although that of tigecycline was superior (MIC50/MIC90, 0.06/0.12 mg/L and 0.25/1 mg/L, respectively).

Conclusion: The finding in our hospital of 2 strains with an MIC of 16 mg/L to metronidazole confirms the need for surveillance of resistance by CD to antibiotics – especially to the drugs of choice – reported in other Spanish hospitals. Alternative drugs should be studied.

P1263 Selection and persistence of erythromycin-resistant Campylobacter coli strains at a pig farm during and after tylosin treatment

P. Juntunen*, H. Heitska, A.L. Myllyniemi, M.L. Hänninen (Helsinki, FI)

Objectives: The use of antimicrobials in production animals is a major cause of antimicrobial resistance in Campylobacter strains. Erythromycin, a macroide group antimicrobial agent, is one of the drugs of choice for the treatment of human campylobacteriosis. Tylosin is a macroide group antimicrobial agent used to treat Lawsonia intracellularis infections in pigs. Our aim was to study selection of antimicrobial resistant Campylobacter coli strains at a farm where pigs are treated with tylosin and persistence of the resistant strains after the treatment.

Methods: Samples were collected from a Finnish pig farm during the treatment of weaned pigs with tylosin between October 2007 and February 2008 (a total of 125 samples from both treated and untreated pigs). Forty eight samples were taken a half year after the ending of the administration of tylosin for weaned pigs in October 2008. Campylobacter were recovered by selective enrichment as well as on CCDA selective medium. One to six Campylobacter colonies were identified from each positive sample (390 colonies in total), and antimicrobial susceptibility to seven antimicrobial agents (erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin, nalidixic acid and ampicillin) was determined from one colony of every positive sample with a microbroth dilution method (VetMIC). In addition, molecular epidemiology of the isolates was followed by PFGE (digestion with Smal).

Results: Of the 173 samples, 145 (83.8%) were Campylobacter positive and of these 145 susceptibility tested isolates, 123 (84.8%) were C. coli and 22 (15.2%) were C. jejuni. All C. coli isolates from the animals not treated with tylosin (n = 33) were susceptible to erythromycin. Of the C. coli isolates collected from the weaned pigs treated with tylosin for four days or longer (n = 49), 49% were resistant to erythromycin (MIC ≥ 32 mg/L). A half year after the ending of the administration of tylosin, 17.0% of the C. coli isolates were resistant to erythromycin.

Conclusion: Tylosin treatment of pigs selected C. coli strains which were resistant to erythromycin. These resistant strains seemed also to persist at the pig farm.

HIV and AIDS

P1264 Syphilis has no virological-immunological interference with the course of HIV disease

R. Manfredi*, L. Calza (Bologna, IT)

Introduction: The reciprocal influence of HIV infection and syphilis are not completely understood.

Patients and Methods: After the recent evidences of a recrudescence of sexually-transmitted diseases (STD) during HIV infection, since the year 2001 we carried out an observational study on a cohort of over 1000 HIV-infected patients (p). Fifty-4 p (36 homo-bisexuals and 18 heterosexuals, aged 23–58 years) were identified as novel cases of syphilis (S) (secondary S in 39 cases, primary or latent disease in the remaining episodes).

Results: All p were assessed and treated based on standardised protocols, and followed for the 12–24 subsequent months. Immunological data including at least 6 months preceding S and at least 9 months following S were available. All p save six took HAART, according to current international recommendations. During the over-18-month observation period, no statistically significant trend of laboratory parameters of HIV disease was seen in our HIV-infected p co-infected with S.

Discussion: Although interactions between S and HIV were not deeply investigated until now, the HIV-related quantitative and functional damage of cell-mediated immunity could modify the course of S. Concurrently, during S an impairment of cellular migration and clearance, and cytokine network, were documented, together with an increased lymphoid cell apoptosis. However, it remains difficult that a non-opportunistic disease like S may trigger pathogenetic mechanisms capable of influencing significantly the HIV disease course, especially when an effective HAART treatment concurs. While we agree with the concerns related to STD in p with HIV or exposed to HIV, differently from literature data (Buchacz K, AIDS 2004;18:2075), in our experience syphilis does not seem to modify the laboratory course of HIV infection. Although health care givers should take into consideration all suspected STD in HIV-infected p, only prospective case-control studies may answer questions associated with the potential existence of bidirectional pathogenetic-clinical interactions between S and HIV infection.

P1265 Misquantifications of HIV RNA level in plasma from a cohort of 100 HIV-1 newly-diagnosed individuals in Marseilles, south-eastern France, as assessed by an “alternative” PCR assay

P Colson*, J. Convert, A. Motte, C. Tamalet (Marseille, FR)

Objectives: Accurate measurements of plasma HIV-1 RNA (VL) is essential for successful clinical management in HIV-1-infected patients. However, the high level of HIV-1 diversity worldwide is an ongoing challenge for primers/probes-based tests, with potential failures of VL measurement. Therefore, objective assessments of the proportion of underquantified VL with current commercial real-time PCR assays are needed in unsorted clinical cohorts. We aimed at assessing the proportion
of severe (>1 Log) VL underquantification using our routine commercial assay among patients in whom HIV infection was newly-diagnosed. **Methods:** 100 patients in whom HIV infection was newly-diagnosed in 2006 were studied. Comparative analysis was performed between VL measured, in the setting of the baseline evaluation panel of HIV infection, with the gag-based commercial Cobas Ampliprep/TaqMan Roche assay used in our routine clinical setting and the ANRS AC1/Biocentric LTR-based Generic HIV viral load assay. Detection thresholds for Roche and ANRS/Biocentric assays were 1.6 and 2.4 Log copies/ml, respectively. In case of VL difference >0.5 Log, VL was retested using both assays on at least one plasma sample. HIV-1 pol sequences were obtained from plasma by direct sequencing with in house protocols. HIV-1 subtype was determined using phylogenetic analysis and the NCBI genotyping tool. **Results:** 57% of patients were male. Mean age was 42±12 years. Mean CD4-cell count was 409±308/mm³. Mean VL was 4.1±1.3 Log copies/ml using Roche assay and 4.5±1.3 using ANRS/Biocentric assay. Mean ANRS/Biocentric VL – Roche VL difference was 0.1±0.7 Log. When excluding plasma samples with VL around or below the detection threshold of the ANRS/Biocentric assay, 3 serum samples showed an ANRS/Biocentric VL – Roche VL difference >1.0 Log (2.7, 1.4, and 1.0 Log). HIV from these 3 plasma samples was classified subtype B in one case, CRF-02AG in another case, and could not be amplified in the third case. One plasma sample showed a Roche VL – ANRS/Biocentric VL difference of 1.0 Log. HIV-1 in this case was classified subtype G. **Conclusion:** Severe underquantification of VL using our routine commercialised assay might involve 3% of patients at time of baseline assessment. The present data question the need for systematic VL first measurement using two different assays. Furthermore, they emphasize the need to set-up reference VL quantification panels in order to test available VL assays.

**P1267** Study on the genotypic resistance of HIV-1 in blood monocytes, CD4 T cells and plasma of HIV-infected individuals


**Objective:** Reservoirs of HIV-1 are a major obstacle to virus eradication and can potentially compromise the success of therapy. Then there is a need to fully understand the molecular nature of the virus population that persist in cellular reservoirs. This study was aimed to characterise the patterns of resistance of HIV-1 in CD14+ monocytes, CD4+ T cells and plasma.

**Methods:** Plasma, CD14+ monocyte and CD4+ T cells were collected from 8 treatment-naïve individuals and 33 treated-patients; of the latter, 10 showed undetectable levels of viraemia and 23 were on virological failure. CD14+ monocyte and CD4+ T cells were isolated using magnetic beads for positive selection (Milteny Biotech). Genotyping of the reverse transcriptase (RT) and protease gene (pro) of HIV-1 was performed using the ViroSeq iPlex method (ViroSeq). HIV drug resistance was defined according to the HIV-1 genotypic resistance interpretation algorithm of the GUIDE LINES TM RULE 12.0-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 in 18 of the 23 “failing” patients (78%) drug resistance mutations were distributed differently from one compartment to another. In only one patient both HIV-1 in monocytes and in CD4+ T cells showed the same pattern of mutations of the virus detected in circulating virus. As far as concern the group of virological suppress patients, sequence analysis was performed only on cell-associated virus, since all individuals showed undetectable level of HIV-RNA (<50 copies/ml). The results obtained revealed that, in 80% of samples, the HIV drug resistant variant harbouring in blood monocytes was different from that archived in CD4+ T cells. Only sequences of drug-sensitive virus were found in both compartments of treatment-naïve subjects.

**Conclusions:** Circulating monocytes may harbour a viral dominant population different from the viruses circulating in the blood and archived in other cellular compartments. HIV-infected monocytes can be an indirect source of HIV-1 by carrying virus and differentiating into tissue macrophage where HIV may productively replicate. Hence, blood monocytes might serve as an indirect source of drug-resistant viral variant.

**P1268** Clinical and virological correlates of HIV-1 central nervous system disease

E. Wey*, A. Geretti (London, UK)

**Objectives:** To categorise and investigate the compartmentalisation of HIV-1 in plasma and CSF with respect to genetic divergence in a cohort of HIV-1 infected patients with varying neurological symptoms and signs.

**Methods:** A search was performed using the WinPath system for CSF/Plasma samples which had been received from HIV positive patients from February 2001–September 2007. 111 CSF samples were identified from 64 patients. For this cohort, data pertaining to CSF Microbiology, CSF Virology, Virological and cellular correlates of HIV disease, arv and clinical history were obtained. Viral loads in CSF and Plasma were measured using the Abbott Realtime instrument. The use of the
Abbott RealTime assay for the quantification of HIV-1 viral load in CSF, was validated using a series of dilutions of HIV-1 RNA made from a standard solution in either negative human plasma or negative human CSF. Patients were classified into subgroups according to log differences between CSF and plasma viral loads and detectability in CSF versus plasma and CSF. In order to establish whether variations in HIV-1 RNA levels or resistance genotypes in CSF were influenced by the variations in the therapeutic levels of antiretroviral medication in CSF and Plasma, samples were sent for Therapeutic Drug Level Monitoring. RNA was extracted using a Biomerieux automated extractor (easyMAG). ViroSeqTM HIV-1 Genotyping System was used to identify mutations in the protease and reverse transcriptase (RT) regions of the pol gene of HIV-1, and sequencing of products was performed using a 3100 Genetic Analyser Instrument. Phylogenetic analysis was performed using MEGA4 software.

**Results:** We have categorised a cohort of 64 patients in which 5 patients demonstrated a 1 log10 difference in HIV-1 RNA viral load between CSF and Plasma, and 5 patients demonstrated 0.5 log10 difference in HIV-1 RNA viral load between CSF and Plasma whilst on HAART. 2 Patients demonstrated identical genotypic resistance profiles in plasma and CSF with no significant compartmental phylogenetic divergence. Data for TDM/sequencing of V3 loop of the gp120 protein pending. (data expected late Jan 2009).

**Conclusions:** An important feature of CNS HIV-1 infection is that its cumulative viral populations can diverge from those in the plasma. Issues pertaining to HAART penetration into the CNS, and bioavailability in CSF need to be considered when interpreting genotypic resistance in both plasma and CSF compartments. Tdm/v3loop data pending.

**P1269 Impact of HLA and related polymorphisms in the control of HIV infection in long-term non-progressors and Elite controllers**

*M. Salgado*, A. Simón, J.L. Vicario, S. Rodriguez-Nocoo, M. Lopez, V. Soriano, B. Rodés (Madrid, ES)

**Background:** The multifactorial mechanisms by which some HIV-1 individuals control infection (long term non-progressors, LTNP) are becoming more complex; in addition to viral and immunological factors, the host genetic background plays an important role in this control. Among the LTNP, a small group of patients “elite controllers” control viral replication. Little is known about this subset, and data emerging on it could further HIV pathogenesis research.

**Methods:** 52 HIV-1 naive patients with different degrees of progression and viral load (VL) were analyzed, of them 34 were LTNP from the HCVII cohort (mean infection: 18.62 years; mean CD4: 657 cells/μL) and 18 were progressors. Several host genes were analyzed in DNA from PBMCs: CCR5 and HLA were typed by PCR. HLA B*92964942 and HCP5 rs2395029 polymorphisms and quantification of CCL3L1 gene copy number were determined using real time PCR. Chi-square and descriptive statistical analysis were performed.

**Results:** Mean VL in LTNP was 2.76 log cop/mL, of them 13 (38%) had undetectable VL for more than 10 years and were considered elite controllers (EC). Mean VL in progressors was 4.16 log cop/mL, none was EC. No significant differences were observed in the frequency of CCR5delta32 nor CCL3L1 copy number in LTNP and progressors (17 vs 8%; 1.17 vs 1.27, respectively), however EC showed a tendency of having lower CCL3L1 copy number. As expected, HLAB5701 and 2705 were more frequent in LTNP. In addition, HLA Cw1203 was also associated with LTNP and only a slight association was observed in the group of EC and HLA Cw0701/02. HCP5 protective SNP was present in heterozygosis in all HLAB5701 positive patients and associated with LTNP (p=0.021). No association was observed between EC and presence of HCP5 SNP. As for rs9264942 SNP, differences between progressors and LTNP were seen when present in homozygosis (41 vs 15%, p=0.043, respectively) but not in heterozygosis. No association with EC was observed. This SNP strongly associated with HLAB*04 and Cw07 (p < 0.0005).

**Conclusion:** HLA plays an important role in HIV infection control in our group of patients due to HLAB*5701, 2705 and HLAB*1203 genotypes were associated with non-progression. The presence of HLA-B*92964942 SNP was more related with progression indicating a possible direct interaction with viral replication other than CTL response. Finally, no significant differences in host genetics have been detected between EC and viraemic LTNP.

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**P1270 Knowledge on sexually transmitted infections of HIV-infected patients**


**Objective:** To verify knowledge about Sexually Transmitted Infections (STI) among HIV infected outpatients attending a Department of Infectious Diseases.
Methods: Cross-sectional survey with a multiple choice questionnaire to HIV infected outpatients attending L. Sacco University Hospital, Milan (Italy) between Feb-Apr 2008. Scores assigned: +1 point for each correct and −1 for each incorrect answer. Unpaired t test and one way Anova (p < 0.05 considered as significant) were used to compare scores among groups.

Results: 428 patient (310 M, 113 F, 5 na) aged 44.6 y (±9.7) accepted to participate. They were Italian (91.2%), single (70.2%), with high level education (86.5%) who acquired HIV infection by sexual exposure (68.1%), with a mean antiretrovirals treatment period of 8.9 y (±6.0); most of them actually on treatment (75%); mean self reported CD4 cell count was 535.2 cells/μL (±260) and HIV RNA log10 2.11 (±0.9).

Clinical, immunological and epidemiological knowledge about HIV infection was high, while the knowledge about other STIs is less widespread. Syphilis is reported as an STI by 91.1% of respondents but only 20.6–29.9% can associate STIs with late complications like sterility or cancer; only 48.6% is able to correctly define gonorrhoea; HSV, HPV, NGU, Trichomoniasis are recognized as STI by a very low percentage of patients.

As primary prevention of HIV/STI condom use is considered useful by 94.2% while monogamy by 33.4% of the patients. Knowledge about vaccine primary prevention is complex and hard to define: HBV vaccine is reported by 61.2%, HAV by 39%, HPV by 22.2%, but vaccine for HCV (25%), HIV (7.2%), syphilis (7.9%), HSV (4%) and gonorrhoea (3.3%) are reported too.

Older ages (>60), lower education level (primary vs secondary school or university), unemployment (vs employment) and suppressed viraemia (HIV RNA log10 <50 copies/mL) are significantly associated to lower scores. No significant association were observed for sex, nationality, marital status, years of antiretroviral treatment and CD4.

Most of the patients (71.7%) declared to need more information especially on HPV associated diseases, other specific STIs, HIV, safe behaviours and vaccine prevention.

Conclusions: Most of HIV positive experienced outpatients had adequate knowledge and appropriate behaviours on HIV infections probably for the high counselling pressure by infectious diseases specialists of the Department. More efforts must be done to extend the body of knowledge also on other STIs.

Figure: Scores of knowledge on HIV/STIs according to age, years on antiretroviral treatment, CD4+ cells and HIV viraemia.

[1271] Prevalence of HIV infection in Saudi Arabia
A. Alothman*, K. Altalhi, A. Al Saedy (Riyadh, SA)

Background: Human immunodeficiency virus infection affects all societies, including the very conservative society. In the beginning of acquired immunodeficiency syndrome (AIDS) era the number of HIV-positive in Saudi Arabia was estimated by UNAIDS with prevalence of 0.2%.

Methods: Obtaining the correct data from the AIDS program at Saudi Ministry of Health according to the annual report of HIV-positive patients.

Results: Between 1984 and 2001, 6046 were found to be HIV-positive, 1285 were Saudis averaging 76 new cases per year. The reported number from the Saudi MOH was 8919 total persons who were HIV-positive at the end of 2004. saudis were 2005 cases. The rate of annual incidence of HIV-infection in Saudis was 275 cases per year. The last 6 years the new HIV infection in Saudis was 1748 cases only between early 2002 and end of 2007. The total number of HIV-positive Saudis on early 2008 was 3033 persons. The calculated HIV-infection prevalence would be 0.02% in Saudi Arabia.

Conclusion: Reporting of HIV-infections in Saudi Arabia is better now compared to the beginning of AIDS era “early 80’s”. The last 6 years showed significant increase in the number of HIV-infected people in Saudi Arabia (1748 cases). This increase in number should be dealt with seriously.
intrapartum and in the newborn is very important and that, statistically, reduces mother-to-child transmission of HIV. These results were similar to other studies done before.

**P1273** Kinetics of expression of activation markers in HIV-infected adults: one-year follow-up
Z. Bartoska, O. Beran, H. Rozsyпал, M. Holub (Prague, CZ)

**Objectives:** HIV infection is associated with chronic immune activation that includes changes of CD38, CD28 and HLA-DR expression on CD8+ T cells, expression of CD154 on CD4+ T cells and CD38 and CD27 on B lymphocytes. These changes are considered as adverse prognostic factors and correlate with the progression of HIV infection. The aim of the study was to analyse the kinetics of expression of these parameters during a year follow-up and to assess the effect of antiretroviral therapy (ART).

**Methods:** 48 HIV+ patients were enrolled in this prospective study including 29 patients on ART (group A), 8 patients prior to ART introduction (group B) and 11 patients without therapy (group C). The control group consisted of 34 HIV- individuals. The following parameters were analysed at the baseline and after 12 months: CD4+ and CD8+ T cell count, B lymphocyte count, HIV-1 viral load (VL) and expression of non-specific activation markers. Immunophenotyping was done using monoclonal Ab and flow cytometry analysis.

**Results:** We observed significantly higher percentages of CD8+CD38+, CD8+CD28-, CD8+HLA-DR+ and CD4+CD154+ T lymphocytes in all groups of HIV+ patients compared to healthy controls. The same trend was found in comparison of non treated HIV+ patients with patients on ART, however only mean fluorescence intensity (MFI) of CD8+HLA-DR+ differed significantly. We observed a significant decrease of expression of CD154 on CD4+ T cells over the study period in all groups. In group B, CD27 expression on B cells was also decreased. Despite higher VL in group C compared to groups A and B after 12 months, the lack of correlation was found between the expression of non-specific activation markers and VL.

**Conclusion:** Our data suggest persistent T cell activation is present in all HIV+ groups when compared to healthy controls. The expression of activation markers on CD8+ T cells in HIV+ patients is not significantly influenced by ART despite the suppressive effect of ART on VL. The observed kinetics of CD154 expression on CD4+ T cells indicates that this marker is the most sensitive for monitoring of chronic immune activation.

**Acknowledgement:** The study was supported by the grants GAUK Nr. 18/06 and GACR Nr. 310/05/H533.

**P1274** Differential risk of lipoatrophy and mitochondrial toxicity among non-Caucasian patients treated at an AIDS centre, Jerusalem

**Objectives:** The choice of NRTI therapy as the optimal backbone for the treatment of HIV patients and carriers is no longer driven exclusively by the differential efficacy of these drugs. Drug toxicity has become a major parameter when antiretroviral regimen is prescribed. Lipoatrophy is one of the chronic side-effects which is clinically manifested as progressive loss of the subcutaneous fat tissue causing significant cosmetic disfiguration. Nolan et al has previously described the effect of different NRTIs upon adipose tissue of Caucasian patients by quantitative measurement of mitochondrial DNA depletion per gram of adipose tissue. The toxicity was most pronounced with Stavudine. Substantial reduction of mitochondrial DNA was also demonstrated in patients treated with Zidovudine.

Since drug toxicity might be related to genetic and ethnic background, these results may not be applicable to other ethnic groups such as African patients. More over, Stavudine is widely used in Subsaharan Africa as part of First-Line regimen, as recommended by WHO.

**Aims:** Cross sectional evaluation of mitochondrial toxicity as induced by Thymidine analogues and non-Thymidine analogues in fatty tissues of non-Caucasian HIV+ Patients volunteers treated at the Hadassah AIDS Center.

**Methods:** Non-Caucasian HIV carrier and Patients treated more than 6 months with Stavudine/Zidovudine or Abacavir/Tenofovir underwent fat tissue biopsy from the iliac crest. Samples were analyses in the Centre for Clinical Immunology and Biomedical Statistics (CCIBS), Royal Perth Hospital, Australia for mitochondrial DNA content per adipocytes.

**Results:** 11 non-Caucasian HIV patients underwent subcutaneous fat biopsy. All the patients has been exposed to at least one Thymidine analogue (Zidovudine-11, Stavudine-2, Diadosine-3). Mean exposure was 50.1 months (3–132 months). The log10 mean mitochondrial DNA content was 2.6 copies/cell (SD 0.4) similar to Caucasian HIV patients treated with Thymidine analogs-2.65 copies/cell (SD 0.36) and much lower than the log10 mitochondrial DNA content in Caucasian HIV patients treated with non-Thymidine analogs-3.05 copies/cell (SD 0.37) and Caucasian HIV naive patients 2.99 copies/cell (SD 0.41).

**Conclusions:** In non-Caucasian HIV patients, Thymidine analogues are as mitochondrial-toxic as in Caucasian patients. This data suggest that Stavudine may need to be dropped as a first line ART regimen in Sub-Saharan Africa, as has been done in the West.

**P1275** HLA-B*5701 screening to avoid hypersensitivity to abacavir
F. Dutly, E. Saller° (Zurich, CH)

**Objectives:** Abacavir is a highly effective reverse transcriptase inhibitor with activity against the human immunodeficiency virus (HIV). However, during the first 6 weeks of treatment up to 8% of patients develop an immunologically mediated hypersensitivity reaction (HSR) with severe symptoms, which reverse only after immediate and permanent discontinuation of abacavir. In 2002, two independent research groups described for the first time a correlation between hypersensitivity to abacavir and carriage of the major histocompatibility complex class I allele HLA-B*5701. The aim of this study was to develop a diagnostic test, which permits accurate screening of the HLA-B locus.

**Methods:** Two primer pairs were designed to specifically amplify the HLA-B*57 gene locus. One pair amplified the entire HLA-B*57 exon 3 while the other one amplified part of exon 2. Subsequently, the amplicons were sequenced and the genotype determined by comparison with the sequences available on http://www.ncbi.nlm.nih.gov/projects/gv/mhc/main.fcgi?cmd=init.

**Results:** The chosen primer pairs were 100% specific allowing discrimination between the HLA-B*57 positive and negative alleles. The HLA-B*57 positive probes were purified and further analyzed by sequencing. The exon 3 sequence was able to recognize the following HLA-B*57 alleles: 0102, 0103, 02, 0301, 0302, 04, 05, 06, 07, 09, 11, 12, 13 and 14. The sequence from exon 2 allowed discrimination between HLA-B*5710, HLA-B*5712, HLA-B*5715 and HLA-B*5716. However, this method allowed no discrimination between HLA-B*570101 and HLA-B*5708, which differ only at the very 3′-end of exon 2.

**Conclusion:** The hypersensitivity reaction to abacavir associates with the locus HLA-B*5701. We developed a diagnostic test, which allows screening of the HLA-B locus through gene sequencing. The advantage of our assay is that we sequence nearly the entire HLA-B*57 gene and thus identify the majority of its alleles.

**P1276** A comparative study of the renal functions in HIV-infected patients treated with or without tenofovir disoproxil fumarate
K. Woratanarat*, C. Suankratay (Bangkok, TH)

**Objectives:** No prospective controlled study evaluating all renal functions in patients receiving tenofovir disoproxil fumarate (TDF) compared with those receiving other nucleoside analogues has been done. Our study aimed to compare the incidence of all renal dysfunctions in

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*S351 HIV and AIDS*
patients receiving TDF and zidovudine (AZT) at King Chulalongkorn Memorial Hospital, Bangkok, Thailand.

Methods: A prospective controlled study evaluating renal functions including creatinine clearance (CrCl) and all tubular functions was conducted in HIV-infected patients receiving either TDF- or AZT-containing HAART regimen from February to December 2008.

Results: Of 29 patients, there were 21 and 8 patients with male to female ratio of 13:8 and 1:1 in the TDF and AZT group. Coadministration with non-nucleoside reverse transcriptase inhibitor or protease inhibitor was noted in 18 (65.5%) and 3 (14.3%) patients in the TDF group, and 7 (87.5%) and 1 (12.5%) patients in the AZT group, respectively. Except for the higher mean CD4 cell count in the TDF group than the AZT group (365.76 and 194.13 cells/mm3, p = 0.011), there were no significant differences in other baseline characteristics. Among the TDF group, the CrCl at baseline, 3 months, or 6 months of treatment was not significantly different. Among the AZT group, there were also no significant differences in the CrCl at baseline, 3 months, or 6 months. At 3 months of treatment, the mean change of CrCl from the baseline (delta CrCl) (-0.5876 mL/min/1.73 m2 and +5.45 mL/min/1.73 m2 in the TDF and AZT group, respectively) between the 2 groups was not statistically different. The delta CrCl at 6 months of treatment between the 2 groups was not different (+2.209 mL/min/1.73 m2 and +2.225 mL/min/1.73 m2 in the TDF and AZT group, respectively). Both CrCl and delta CrCl between the 2 groups were still not different even the calculation was based on MDRD Study Equation and Cockcroft-Gault formula. Ammonium chloride loading test to assess the proximal tubular function showed no abnormalities in the 2 groups. Diabetes insipidus, acute renal failure, and Fanconi syndrome were not observed during the study.

Conclusion: To the best of our knowledge, this is the first prospective controlled study comparing all renal functions in HIV-infected patients receiving TDF or AZT. No differences in the incidence of renal failure and renal tubular dysfunction between the 2 groups. However, we need to confirm our observation with longer period of treatment.

P1277 Use of tenofovir disoproxil fumarate and monitoring of renal function among HIV-1-infected patients in a resource-limited setting

P. Deangchalermsong, S. Sungkamupaph*, S. Watcharananan, K. Malathum (Bangkok, TH)

Objectives: Tenofovir disoproxil fumarate (TDF) has recently been available in Asia and renal dysfunction in patients receiving TDF has been reported. This study was aimed to evaluate the use of TDF and the monitoring of renal function among HIV-1 infected patients in a resource-limited setting.

Methods: We evaluated the use of TDF in a cohort of HIV-1 infected patients who were initiated TDF either in antiretroviral-naive or -experienced patients. Reasons for using TDF were determined and methods and compliance of monitoring for renal function were assessed. Estimated creatinine clearance (CrCl) by Cockcroft-Gault calculation was used.

Results: We studied 205 patients with a mean (SD) age of 44.3 (9.4) years and 64% male. Mean (SD) body weight was 58.5 (10.5) kgs. Median (IQR) CD4 cell count were 389 (289-514) cells/mm3. Of all, 22% had HBV co-infection and 8% had HCV co-infection. Prior to initiation of TDF, serum creatinine (Cr) and urinalysis were tested in 183 (80%) and 44 (21%) patients, respectively. At baseline, mean (SD) CrCl was 85.7 (23.3) mL/min and only 1% of patients had Cr > 1.5 mL/dl; 4% of patients had proteinuria. Reasons for initiation of TDF included lipodystrophy from d4T and/or AZT (51%), regimen simplification (20%), HBV co-infection (13%), virologic failure (10%), and adverse events from other NRTIs (6%). Regarding antiretroviral regimens, 71% of patients used TDF in NNRTI-based regimens while the others used in PI-based regimens. Lamivudine was the most common NRTI used together with TDF (86%). After initiation of TDF, 58% of patients had been followed up for serum Cr at a median (IQR) duration of 4 (2-7) months after initiation of TDF; mean (SD) CrCl was 82.7 (25.3) mL/min and 3% of patients had Cr > 1.5 mL/dl. Both CrCl and Cr were not significantly different from baseline (p > 0.05). From linear regression, only baseline Cr was associated with CrCl at follow-up after TDF initiation (β = 0.844, p < 0.001). Other factors including age, gender, weight, plasma glucose, and concomitant PI use were not associated with CrCl after TDF initiation (p > 0.05).

Conclusion: In resource-limited setting, TDF is commonly used for substitution of d4T or AZT secondary to lipodystrophy. It appears that assessment of renal function prior to initiation of TDF and monitoring of renal function after initiation of TDF are inadequate. Baseline Cr is a good predictor for CrCl change after initiation of TDF; it should not be omitted in resource-limited setting.

P1278 Long-term risk of pneumocystosis after earlier discontinuation of prophylaxis among HIV-infected patients receiving highly active antiretroviral therapy


Background: Primary or secondary prophylaxis for pneumocystosis can be safely discontinued in HIV-infected patients when their CD4 counts increase to 200 cells/μL after receiving highly active antiretroviral therapy (HAART). However, a substantial proportion of the patients may have to discontinue prophylaxis earlier due to adverse effects of antimicrobial prophylaxis. Long-term risk of pneumocystosis after discontinuation of primary or secondary prophylaxis among HIV-infected patients before CD4 counts increase to ≥200 cells/μL after HAART is rarely investigated.

Methods: Between 1 April, 1997 and 30 September, 2007, 660 HIV-infected patients who had baseline CD4 counts <200 cells/μL and had been followed up for more than 3 months after HAART were enrolled in a prospective observational study to examine the incidence rates of pneumocystosis when primary or secondary prophylaxis for pneumocystosis was discontinued before CD4 counts increased to ≥200 cells/μL after HAART.

Results: Of 521 patients who did not initiate any antimicrobial prophylaxis (n=165) or discontinued primary or secondary prophylaxis for pneumocystosis before CD4 counts increased to ≥200 cells/μL (n=356) after HAART, 21 cases of pneumocystosis developed after a total observation duration of 1810 persons-years [PY], with an incidence rate of 1.16 per 100 PY (95% confidence interval [CI], 0.71, 1.77). Of 139 patients who continued primary or secondary prophylaxis until CD4 counts increased to ≥200 cells/μL after HAART, 3 cases of pneumocystosis developed after a total observation duration of 445 PY, with an incidence rate of 0.66 per 100 PY (95% CI, 0.13, 1.93). Compared with the latter group of patients, the risk ratio of developing pneumocystosis and all-cause bacterial infections for the former group after earlier discontinuation of antimicrobial prophylaxis was 1.765 (95% CI, 0.5238, 5.886) and 1.58 (95% CI, 0.9859, 2.533), respectively.

Conclusions: Long-term risk of pneumocystosis was low among HIV-infected patients who discontinued primary or secondary prophylaxis before CD4 increased to ≥200 cells/μL after receipt of HAART with favourable virologic and immunologic responses.

P1279 Serological screening of Chagas' disease in HIV-positive immigrants proceeding from endemic areas in Spain

A. Rodríguez-Guarda*, V. Asem, M. Rodríguez Pérez, P. Mejuto, P. Alonso, J.A. Cartón (Oviedo, ES)

Background: Chagas’ disease is an opportunistic infection in the setting of HIV/AIDS. The arrival of HIV positive immigrant proceeding from endemic areas to non-endemic countries makes possible the detection of Chagas’ disease in this group of patients. We describe the results of a screening program conducted in HIV + immigrant population coming from endemic areas.

Methods: We determined anti-T. cruzi antibodies in all HIV patients proceeding from endemic areas in follow-up at the Infectious Disease
Unit of Hosp. Central of Asturias, during 2007. The ID-Chagas antibody test (Particle Gel Immuno Assay – PaGIA, DiaMed-ID-) was used as screening assay. The positive cases were confirmed with a second ELISA (Total Ag) and a nested PCR. In all the confirmed cases a protocol that included a clinical-epidemiological evaluation, chest X ray, EKG, esophagogastroscopey, barium enema, and echocardiography was applied.

Results: We screened 19 HIV positive immigrants (mean age 36 years). The procedure countries were: Brazil and Ecuador (26% each), Colombia (21%), Paraguay, Uruguay, Argentina, Dominican Republic and Bolivia (5.2% cases each). Two patients (10.5%) had positive antibody test for Chagas’ disease, that was confirmed in both cases. PCR was positive in both cases. Direct microscopic examination of blood was negative in both. The positive patients were a man coming from Bolivia and a woman from Paraguay. Both lived in houses where the reproduction of triatomine bugs is possible. Both were on HAART with CD4 of 348 and 456 CD4+ cells/mm3 and with HIV-1 RNA <10 copies/ml. Both patients were asymptomatic and had normal additional studies for Chagas’ disease.

Conclusions: The overlap of HIV and T. cruzi infection occurs not only in endemic areas but also in non-endemic areas of North America and Europe where the diagnosis may be even more difficult. It is necessary the realisation of screening programs in this group of population for the early diagnostic of Chagas’ disease.

S353

P1281

Parovirus B19 and Erythrovirus type 2 and 3 infections are infrequent in HIV-infected patients with CD4 <500 cells/mm³ and chronic anaemia

T. Ferry, B. Hitschel, T. Dang*, P. Meylan, A. Rauch, R. Weber, L. Elzi, E. Bernasconi, P. Vernazza, A. Calmy on behalf of the Swiss HIV Cohort Study

Background: Erythroviruses, Parovirus B19 (PV B19) and the newly described Erythrovirus genotype 2 and 3, mainly target human erythroid progenitors. The pathogenic role of PV B19 infection is strongly influenced by the haematologic and immunologic status of the host. In immunosuppressed patients, a reactivation of PV B19 may occur and may lead to severe acute or chronic anaemia and zidovudine ( AZT) intolerance. The pathogenic role of newly described Erythroviruses is unknown.

Objectives: We aimed at screening for Erythrovirus replication in a large cohort of HIV-infected patients presenting with CD4 cell count <500 cells/mm³ and chronic anaemia to test if patients with HIV infection were at risk for symptomatic parvovirus infection.

Methods: Patients included in the Swiss HIV Cohort study from 1998 to 2007 were selected according to the following criteria: (i) persistent anaemia (haemoglobin [HB] level below 10.5 g/dL during at least three months) (ii) CD4 cell count <500 cells/mm³ during the episode of anaemia and (iii) availability of at least one frozen serum or plasma sample during the period of anaemia. Detection and quantification of Erythroviruses was performed with a real-time PCR targeting the VP1 gene, a well conserved region of the Erythrovirus genomes.

Results: 428 patients were included in the study (median age 44 years, female sex 61%, intravenous drug user 36%, median CD4 cell count 187 cells/mm³, median Hb level 9.5 g/dL, AZT exposure 41%); circulating Erythrovirus DNA was detected in 16 of them. Viral load ranged from 18 to 6820 copies/mL and was low (<500 copies/mL) in 13 patients. No differences were noticed after comparison of patients with or without Erythrovirus replication with regards to route of transmission, CD4 cell count and AZT exposure.

Conclusion: Erythroviruses infections appear to be an infrequent finding in HIV-infected patients presenting with low CD4 cell count and chronic anaemia, despite the use of an ultrapsensitive PCR technique

P1282

Non-AIDS defining malignancies in the era of combined antiretroviral therapy

R. Manfredi* (Bologna, IT)

Introduction: The introduction of combined antiretroviral therapy (cART) since the year 1996, contributed to a rapid, significant drop of frequency of all AIDS-defining opportunistic infections and some selected AIDS-related tumours (like Kaposi’s sarcoma) with a consequent, remarkable reduction of both morbidity and mortality rates associated with these disease complications.

Patients and Methods: Our cohort of over 1700 HIV-infected patients followed in two centred outpatient centres by the same physician staff were prospectively followed since the year 2000 (8 years), with special interest focused on the diagnosis, treatment and outcome of non-AIDS related malignancies.

Results: Among haematological malignancies other than non-Hodgkin’s lymphoma and primary central nervous system lymphoma, we observed three cases of acute myelogenous leukaemia and 2 episodes of Hodgkin’s lymphoma. A greater number of solid tumours involved different organs and sites: laryngeal cancer (5 cases, with 3 episodes of papillomatous laryngeal cancer), rhinopharyngeal squamous carcinoma (2 cases), adenocarcinoma of the lung (3 cases), gastric adenocarcinoma (2 episodes), oesophageal carcinoma (one patient), prostate cancer (3 cases), bladder adenocarcinoma (2 episodes) pancreatic adenocarcinoma (one case), and pheochromocytoma (one episode). Some of these malignancies have been reported with extremely rare frequency until now (usually as single-case anecdotal reports), in particular before the cART era. The patient’s age ranged from 34 to 67 years, the
mortality rate of these last 23 episodes was very elevated (78.3%),
and occurred 4–38 months after diagnosis, despite appropriate surgical
and/or cytotoxic chemotherapy.

Discussion: The significantly increased life expectancy of HIV infected
patients in the cART era was characterised by a proportionally increase
of non-AIDS-defining tumours, which may depend on the advanced
mean patients’ age, their prolonged exposure to risk factor, the
persisting functional immune system imbalance, and probably some
direct oncogenic property of HIV itself, even when a “quantitative”
recovery of CD4+ lymphocyte count has been achieved thanks to cART.
The differential diagnosis of non-AIDS-associated tumours may be
delayed by the low clinical suspicion, and their frequency to mimic
and/or overlap infectious complications. Further epidemiological and
clinical investigation is strongly warranted to increase the awareness
of this emerging phenomenon.

P1285 Long-term effects of occult hepatitis B virus infection in
human immunodeficiency virus infected patients
(Shanghai, CN)

Objective: To determine the clinical significance of occult hepatitis B
virus (HBV) infection in human immunodeficiency virus (HIV)-infected
patients.

Methods: We performed a retrospective cohort study among HBV sur-
face antigen negative, hepatitis C virus antibody negative, HIV-infected
patients. Their serum samples obtained before the beginning of highly
active antiretroviral treatment (HAART) were used to amplify surface
protein region of HBV previously. Patients with HBV DNA positive
were classified as occult HBV co-infection. Hepatic transaminase levels
and CD4 T cell counts were collected at 0.5 or 3-month intervals from
the beginning of HAART. The incidence rate of hepatic flare was assessed
by the chi-square test or Fisher’s exact test. A P value less than 0.05 was
considered to be statistically significant.

Results: 11 and 21 patients enrolled as occult HBV co-infection
group and HIV mono-infection group respectively were followed up for
21.9±6.7 months. CD4 T cell count increased 178.9±22.1 cell/mm³ and
146.6±21.9 cell/mm³ in occult HBV co-infected group and HIV-mono-
infected group after one year of HAART, respectively. And there was no
significant difference between these two groups (P=0.35). During
follow up, 72.7% (8/11) patients occurred at least once hepatic flare in
the occult HBV co-infection group, it was not statistically different when
compared to 52.4% (11/21) patients in the HIV mono-infected group.
However, when excluding the first 3 months of HAART period during
which hepatic flare occurred frequently because of antiviral drug side
effects and immune restoration syndrome, this event was more frequent
in occult HBV co-infected group than in HIV mono-infected group
(63.6% versus 22.7%, P=0.02).

Conclusions: Occult HBV infection had no impact on response to
HAART regarding immune recovery in HIV/AIDS patients. However, it
can increase the incidence of hepatic flare in HIV/AIDS patients. Future
studies should determine whether occult HBV infection is associated
with other clinically important outcomes, particularly hepatocellular
carcinoma.

P1286 The association between changes in depression and
adherence to highly active antiretroviral therapy among
adult HIV-infected patients in Thailand
N. Hirasansithikul*, N. Buathong, S. Tangwongchai, C. Komoltri
(Bangkok, TH)

Background: Non-adherence to highly active antiretroviral therapy
(HAART) leads to development of HIV resistance mutation and
treatment failure. Depression is a common psychological disorder among
HIV-infected patients.

Objectives: To evaluate the association of depression and cofactors with
adherence to HAART in Thai adult HIV-infected patients and to assess
the temporal association between changes in depression and adherence
to HAART.

Material and Method: A concurrent cohort study was conducted at
King Chulalongkorn Memorial hospital from October 2007 to April
2008. A total of 379 HIV-infected patients were recruited. Participants
completed seven questionnaires regarding to demographic and patients’
medication characteristics, adherence to HAART, depression status,
cognitive function, alcohol use disorder, HIV social support and
HIV-related physical symptoms.

Results: The rates of non-adherence to HAART among studied
participants at week 4, 8 and 12 were 34.6, 19.9 and 24.1 percents.
The prevalences of depression at baseline, week 4, 8 and 12 were 33.3,
32.2, 23.8 and 27.9 percents respectively. In longitudinal multivariate
analysis, statistically significant factors associated with non-adherence
were depression (mild; adjusted OR=16.03, 95%CI = 6.73–38.17,
moderate to severe; adjusted OR=7.40, 95%CI = 3.10–17.64), alcohol
use disorder (adjusted OR=4.85, 95%CI = 1.75–13.46), physician’s
not reminding the patients for adherence to HAART during clinic
visit (adjusted OR=9.72, 95%CI = 2.99–31.61), no past history
of opportunistic infection (adjusted OR=2.19, 95%CI = 1.08–4.46)
and using herb (adjusted OR=3.08, 95%CI = 1.09–8.67). Changes in
depression were significantly associated with non-adherence to HAART
(mild; OR = 3.71, 95%CI = 1.86–7.37, moderate to severe; OR = 7.20,
95%CI = 2.74–18.94).

Conclusion: Depression was common among HIV-infected patients and
was a significant predictor to non-adherence to HAART. Physicians who
provide HAART should screen and look for depression in HIV-infected
patients and regularly emphasize the goal of HAART and the importance
of adherence to the patients.
Non-invasive assessment of liver fibrosis by measurement of stiffness in HIV-infected patients. The significance of the metabolic syndrome

H. Sambatakou* (Athens, GR)

Background/aims: There is emerging evidence that severe liver disease may develop in HIV patients. The aetiology is multifactorial and not completely understood, but associations with viral hepatitis co-infection, fatty liver or HAART exposure, especially to didanosine, have been suggested. In this study we estimated the prevalence of liver fibrosis with transient elastography and determined its predictors in a cohort of HIV patients.

Methods: We included 133 HIV patients, including 8 HIV seropositive patients with HBV or HCV co-infection, who are followed in our unit. Liver fibrosis was evaluated with transient elastography (Fibroscan) and a liver biopsy in those patients with a definite diagnosis of liver fibrosis. All patients also underwent liver ultrasonography for the detection of fatty liver. Detailed epidemiological, anthropometrical and laboratory data, including CD4 counts and HIV RNA levels, were recorded. Insulin resistance was assessed by the HOMA index.

Results: Patients' characteristics were: males 91%, mean age 39 years, median BMI 24.9 kg/m². In total, 26 (19.5%) patients had fibrosis and 65 (49%) ultrasonographic evidence of fatty liver. Ninety-two (69%) patients had received anti-HIV treatment for a median duration of 6.5 years. In univariate analysis, presence of fibrosis was associated with increasing age (P = 0.006) and BMI (P = 0.026), high levels of ALT (P = 0.043) and GGT (P = 0.014), presence of hypertension (P = 0.011), abnormal waist circumference (P = 0.009) presence of metabolic syndrome (P = 0.007) and viral hepatitis co-infection. No association was noted between fibrosis and fatty liver, insulin resistance, treatment regimens or treatment duration. In multivariate analysis, the only factors independently associated with fibrosis were metabolic syndrome (OR 5.675, 95% CI 1.253–25.705; P = 0.024), increasing age (OR 1.059, 95% CI 1.001–1.121; P = 0.048) and HBV or HCV co-infection. In the subgroup of 53 patients without ultrasonographic evidence of fatty liver, 7 had elastographic evidence of fibrosis, but no predictive factor could be found.

Conclusions: Liver fibrosis may be found in approximately one fifth of HIV-patients and is independently associated with presence of metabolic syndrome, increasing age and HDV or HCV co-infection. Therefore, such patients may be benefit from therapeutic intervention aiming to the management of metabolic syndrome parameters.

Response to anti-retroviral treatment at an AIDS centre, Jerusalem: comparison between Israeli and non-Israeli HIV+ patients


Background: Urokinase-type plasminogen activator receptor (u-PAR) is a protein that converts plasminogen to plasmin that is elevated in multiple inflammatory processes. Its soluble receptor, su-PAR (soluble urokinase-type plasminogen activator receptor), is a secreted surface protein expressed on macrophages, neutrophils and endothelial cells. High serum suPAR level has been previously suggested to predict poor outcome in malaria, pneumococcal pneumonia, streptococcal infections, tuberculosis and HIV.

Aims: Evaluation of the correlation between suPAR levels, CD4, viral load (VL) and death in untreated HIV patients, as means of simplifying the laboratory follow-up in HIV.

Methods: suPAR, viral load and CD4 levels were determined in serum samples of 146 untreated Ethiopian HIV+ patients (the ENARP cohort) that were followed between 1997–2005 in Addis Abebe.

Results: No significant correlation was found between suPAR levels and parallel CD4 or VL in each time point of follow up. Baseline suPAR or suPAR change during the first year did not predict CD4 or viral load changes during the first year or the first three year of follow up. The baseline suPAR of patients that died during the follow up, was not significantly different from the baseline suPAR level of patients that survived during the follow up p = 0.431, but the last suPAR measurement before death was significantly higher than the last suPAR measurement of patients that did not die 5.6 vs 3.6 p = 0.001. The last suPAR measurement was found to be a predictive parameter of death using Logistic regression model (p = 0.003)

Conclusions: Serum suPAR levels cannot be used as a laboratory marker replacing VL or CD4 since there is no correlation between suPAR levels and these important and predictive parameters, but a dramatic increase during the follow up in suPAR can indicate an impending death.
Methods: Medical files of 134 patients who started anti-retroviral treatment (ART) during 1995–2006 were reviewed. CD4 & viral load at presentation, start of treatment and every 6 months thereafter were recorded as well as dates of first undetectable viral load and date of escape from Rx. Prevalence and pattern of resistance was analyzed. Response to ART was evaluated using repeated measure analysis, T-Test and Mann Whitney test.

Results: Eighty-one (60.4%) of the participants were Ethiopians immigrants, 53 (39.6%) were non-Ethiopians. Median follow-up time was 5.5 years. After 9 years of treatment mean CD4 count in the Ethiopian group (408±190.1 cells/ml) was lower than that in non-Ethiopians (616±391.4; p = 0.125). Exposure to NNRTI's and PI's was comparable in the two patient groups. More Ethiopians were given D4T while more non-Ethiopian were given DDC. The prevalence of primary resistance mutations was similar in both groups (25.9% Ethiopian vs. 30.2% non-Ethiopian, p = 0.8). Analysing as group mutant prevalence of non-TAMs (M184V K65R L74V), TAMs (M41L D67N K70R, L210W T215F) and PI mutations (D30N, L90M, M46I) was similar among Ethiopians & non Ethiopians. The prevalence of NNRTI mutations (K103N, Y188L, V106M, L100I) was higher in the Ethiopian group (42.9% vs 17.6% p = 0.096).

Conclusions: Ethiopian and non-Ethiopian HIV/AIDS patients treated in Israel respond similarly to antiretroviral therapy to, despite minor differences in patterns of HIV mutation. Overcoming cultural, language and adherence difficulties in treatment of this patient group may be partially attributed to case manager involvement, improving patient compliance and understanding.

**P1290 Immunological and virological outcome of a double-boosted HIV protease inhibitor salvage regimen**


Objectives: Double boosted protease inhibitor (PI) combinations have been used as part of salvage regimen for HIV-infected patients. We conducted a retrospective study to assess the immunological and virological outcome of this strategy in a clinical setting.

Methods: All patients followed in two French hospitals and receiving a double PI regimen as part of HAART salvage regimen between 2002 and 2006 were included in the study. Double PI regimen was chosen according to genotypic resistance. CD4 T cell count and HIV-1 RNA viral load (VL) were monitored. Intention to continue treatment analysis was performed.

Results: Seventy-three highly pre-treated patients initiating a double boosted PI regimen (lopinavir/ritonavir + amprenavir: n=35, amprenavir + saquinavir/r: n=16, saquinavir + nelfinavir/r: n= 5, others: n=11) were included. Enfuvirtide was co-prescribed in 4 cases. Baseline median CD4 count and VL were 207/mm³ [interquartile range IQR: 114–318] and 4.3log10 copies/ml [IQR: 3.1–5.0], respectively. Patients had a median of 9.2 years (IQR: 6.8–10.8) of previous treatment and had received a median of 9 antiretroviral drugs (IQR: 6–11) and 2 PI (IQR: 2–4). Median number of total and major IAS PI resistance mutations were 7 (IQR: 3–10) and 2 (IQR: 0–3), respectively. Median follow-up was 18.2 months (IQR: 9.6–26.4); 6 and 4 patients interrupted the double boosted PI regimen before 6 months and 12 months, respectively for virological failure (n = 5) and toxicity (n = 5). No severe adverse event was observed. Kaplan-Meier 6-month and 12-month probabilities of viral suppression (VL < 50 copies/ml) were 46.2% (95%CI = 35.5–58.5) and 64.6% (95%CI = 53.3–75.8). Kaplan-Meier 6-month and 12-month probabilities of an increase of CD4 cell count ≥ 100 cells/mm³ were 39.9% (95%CI = 29.5–52.4) and 53.8% (95%CI = 42.1–66.3).

Conclusions: Double boosted PI combinations were well tolerated and resulted in significant CD4 rise and VL decline in highly pre-treated patients. This strategy may be an alternative for those patients with limited therapeutic options in resource-poor settings, where new expensive antiretroviral classes are not currently available.
Antifungal resistance

Mean follow-up was 34 months (r 1–63). After transplantation ART was re-started at day 17 as average (r 7–52) and currently ART is based on efavirenz in 15 patients, on PI in 2 and on NRTIs in 3 patients. Post-OLT mean CD4 count was 371 cell/mm³ (r 22–667) and plasma HIV-1 RNA was <200 copies/ml in 87% patients. Acute cellular rejection rate was 19%. One patient was retransplanted on day 6 due to a graft massive necrosis. One patient died on day 6 due to an upper gastrointestinal haemorrhage. Late mortality (>6 months) occurred in 4 patients: HCV recurrence in 2 and de novo tumour in other 2. Eight patients have been treated with Peg-Inf plus ribavirin and 5 achieved sustained viral response. Two patients developed opportunistic infections after OLT: gastrointestinal CMV at month 4 and pulmonary tuberculosis at month 8.

Conclusions: Liver transplantation in HIV-infected patients have a good mid-term survival. HIV infection can be adequately controlled after OLT. In our experience, mortality after OLT in HIV-infected patients was mainly related to HCV recurrence and de novo tumours.

Antifungal resistance

Genetic profiles of HIV-1 populations in patients on failing abacavir/lamivudine/zidovudine

S. Hong*, Z. Camacho (Durham, US)

Objectives: To understand evolution pathway of multi-drug resistant HIV-1 virus under drug selection pressure.

Methods: Plasma from baseline and at different intervals post treatment failure was used for RT-PCR from seven patients. To better represent viral quasispecies, the partial pol gene were amplified and cloned in five independent reactions. Multiple clones (9–49) were sequenced for each time point.

Results: Drug resistant mutations were detected in five patients post treatment failure. Phylogenetic analysis showed that viral sequence at different time point clustered separately by forming independent lineages. The genetic diversity was decreased from 1.6% to 0.6% in treatment failure viral populations, while non-synonymous/synonymous mutation ratios increased from 0.067 to 0.118, respectively. In one patient, a baseline subcluster was identified M184V mutation but could not be separated from post treatment population phylogenetically. No known drug resistance mutations were detected in two other patients and phylogenetic analysis showed very homogenous viral population between baseline and treatment failure viruses.

Conclusions: These data suggests that the HIV-1 virus population changed dynamics under continual drug selection pressure. The virus population clustered in a timepoint-specific manner with genetic diversity decreased consistently.

Discordant genotypic interpretation and phenotypic role of protease mutations in HIV-1 subtypes B and G

A. Santos, A. Abecasis, A.M. Vandamme, R. Camacho, M. Soares* (Rio de Janeiro, BR; Leuven, BE; Lisbon, PT)

HIV-1 group M is classified into nine different subtypes. Antiretroviral (ARV) drugs have been developed for subtype B, and the response of non-B subtypes in terms of susceptibility and the acquisition of drug resistance when facing those drugs is largely unknown. In this study, we wanted to address differences in the impact of protease inhibitor-selected mutations in subtypes B and G. ARV-treated, HIV-positive patients regularly followed at the Hospital de Égäes Moniz, in Lisbon, Portugal, were examined for the presence of PI-associated primary mutations (301 of subtype B and 184 of subtype G), and for the selection of those mutations over time of protease inhibitor exposure. Forty-three subtype G mutations were phenotyped for susceptibility to all available PI through VIRCOS’s Antivirogram®, and compared to a similar dataset of subtype B patients. Mutation 154V/L was selected by nelfinavir in subtype G isolates, a mutation not previously described for that drug in subtype B. L90M was associated with a lower reduction in susceptibility of subtype G to nelfinavir when compared with subtype B, and with no reduced susceptibility to saquinavir. This was compensated by the acquisition of M89I in subtype G. L90M did not reduce susceptibility of subtype G to saquinavir, contrarily to subtype B. Likewise, the pattern I54V/L-L90M did not reduce susceptibility of subtype G to indinavir and saquinavir. Indinavir-associated mutations M46I/L, I84V and V82A/F/T developed earlier in subtype B across length of exposure to that drug when compared to subtype G counterparts. Our results provide proof of principle and support the growing evidence that subtype-specific responses to ARV exist. Data presented here highlights inconsistencies in current genotyping interpretation algorithms inadequately applied to all HIV-1 subtypes.

Efficacy of voriconazole against three clinical Aspergillus fumigatus isolates with mutations in the cyp51A gene

E. Mavridon*, J.W. Mouton, E. Mellado, W.J.G. Melchers, P.E. Verweij (Nijmegen, NL)

Objective: Azole resistance in Aspergillus fumigatus (Af) has been associated with substitutions in the cyp51A gene. These substitutions cause different susceptibility profiles and it is unclear if the in vitro activity corresponds with in vivo efficacy. We investigated the correlation between in vitro activity of voriconazole (VCZ) and in vivo survival of clinical Af isolates with L98H, G54W, and M220I substitutions.

Methods: In vitro activity of VCZ was determined based on the CLSI M-38A method. A total of 16 groups (n = 11/group) of CD-1 mice, were randomised into 4 groups for the 4 different Af isolates and were infected i.v. through the lateral tail vein. Oral therapy with VCZ at 80, 40, and 10 mg/kg once daily was begun 24 hours post challenge for 14 days. Control groups received saline orally. Mortality data was analyzed by the log rank test. Survival was determined daily until day 14. Results were analyzed by survival curve analysis and plotting dose/MIC against survival and fitting the Hill equation with variable slope (HEVS) using Graphpad Prism 5.0.

Results: The MICs of VCZ were: 0.25 mg/L (WT and M220I), 0.125 mg/L (G54W) and 2 mg/L (L98H). The median survival time for the control groups for all strains was 2–3 days. VCZ treatment improved survival of the L98H M220I and G54W groups compared to controls (p < 0.001). However, compared to WT and M220I, the dose-response curve of mice infected with the G54W and L98H isolates were shifted to the right indicating that a higher dose was required to achieve maximum response against these strains (R2 of 0.5034, EC50 of 58, 71, Hillslope of 1, 338). While the 40 mg/kg dose revealed 100% survival against the M220I, G54W and WT isolates, only 50% survival rate could be reached against the L98H. There was an excellent correlation between MIC and in vivo efficacy of VCZ.

Conclusions: VCZ showed good efficacy in mice infected with all the 4 isolates when the administered daily dosage exceeded 10 mg/kg. Compared to the other 3 isolates, a twofold increase of VCZ dosage was required for mice infected with L98H to achieve the same maximum response. The in vivo efficacy of VCZ corresponded with the MICs.

Development ofazole resistance in a clinical A. fumigatus isolate with no mutations in the cyp51A gene

M.C. Arendrup*, K.L. Mortensen, W. Melchers, N. Frimodt-Moller, H. Khan, P. Verweij (Copenhagen, Esbjerg, DK; Nijmegen, NL)

Objectives: A. fumigatus triazole clinical resistance has been linked to cyp51A mutations with or without a concomitant tandem repeat in the gene promoter. We report the isolation over a 2.5 year period of 4 sequential A. fumigatus isolates from a CGD patient eventually failing azole and echinocandin combination therapy. The isolates were investigated phenotypically and genotypically and the in vivo efficacy of antifungal drugs was evaluated.

Methods: Susceptibility testing was performed using the EUCAST E.DEF 9.1 microdilution method for conidia-forming moulds. The entire coding region of the cyp51A gene from a susceptible and resistant
A. fumigatus isolate was sequenced. Genotyping was performed using microsatellite typing. The in vivo efficacy of antifungal agents was investigated in an immunosuppressed haematogenous model using NRMI mice weighing 26–30 g. The mice received intraperitoneal injection of 200 mg/kg cyclophosphamide, day -3 and 100 mg/kg day 0 to obtain prolonged neutropenia. Mice were inoculated i.v. with 0.2 ml of a105 CFU suspension day 0 and subsequently treated once daily at days 1−4 and 7−10 with either saline (control), anidulafungin (AND) 12mg/kg, posaconazole (POS) 20mg/kg, or both. Kidney burden and mortality were evaluated day 4, 8 and 11 of mice in groups of 6 or 10. The experiments were approved by the Danish Animal Experimentation Committee under the Ministry of Justice (number 2004/561–835).

Results: The experiments were approved by the Danish Animal Experimentation Committee under the Ministry of Justice (number 2004/561–835).

The POS MICs of the 4 consecutive isolates were 0.125 μg/ml, 0.125 μg/ml, 0.5 μg/ml, and 1 μg/ml, respectively. Caspofungin and AND MICs were stable (range 0.25−0.5 and 0.5−1 μg/ml, respectively). Genotyping showed the isolates were genetically identical and thus of clonal origin and sequencing of the Cyp51 genes revealed no mutations in the hot spots of the gene or its promoter. In the animal model AND alone and AND-POS combination therapy significantly reduced mortality (P < 0.0001) and kidney burden day 8 (P = 0.0121 and 0.0167, respectively) while POS mono therapy did not (P = 0.0856 comparing mortality and P = 0.1167 comparing kidney burden to control group).

Conclusion: This A. fumigatus isolate obtained from a patient failing 2 years of caspofungin–micafungin combination treatment followed by 2 months of POS-caspofungin treatment showed POS resistance in the mouse model despite not possessing previously described resistance mechanisms. AND alone or in combination with POS was effective against azole resistant aspergillosis.

**P1298 In vitro antifungal activity of isavuconazole against 345 mucorales isolates, collected at eight study centres worldwide**

T. Peléz*, G. Gonzalez, N.P. Wiederhold, C. Lass-Flörl, P. Warn, R. Heep, M.A. Ghannoum, J. Guinea (Nijmegen, NL; Nuevo León, MX; San Antonio, US; Innsbruck, AT; Manchester, UK; Basel, CH; Cleveland, US, Madrid, ES)

Background: Aspergillus nidulans is an infrequent but potential cause of invasive aspergillosis. However, its in vitro antifungal susceptibility profile has been poorly evaluated. We studied the antifungal susceptibility of an updated panel of 8 antifungal drugs against 63 clinical strains of A. nidulans collected in our hospital since 1997.

Methods: The strains were from respiratory samples (n = 55), superficial samples (n = 5), and other samples (n = 3) and were identified according to morphological characteristics. Antifungal susceptibility to amphotericin B, terbinafine, caspofungin, micafungin, itraconazole, voriconazole, posaconazole and isavuconazole was obtained following the CLSI M38-A procedure and the results analyzed using the t test and Pearson correlation coefficient (PC).

Results: The antifungal susceptibility (MIC50, MIC90, geometric mean and ranges, in μg/mL) was as follows: amphotericin B (4/4.3/3.1−8), terbinafine (0.062/0.125/0.09/0.031−1), caspofungin (0.125/0.25/0.38/0.062−8), micafungin (0.062/0.062/0.065/0.062−0.125), itraconazole (1/2/1.036/0.25−4), voriconazole (0.5/0.2/0.61/0.062−2), posaconazole (0.5/0.1/0.54/0.025−1) and isavuconazole (0.25/1/0.46/0.062−1). Amphotericin B showed the lowest activity (52.3% of strains with MICs > 4 μg/mL) (P < 0.001). In contrast, terbinafine showed good activity (98.4% of strains with MICs ≤ 0.25 μg/mL). Micafungin showed higher activity than caspofungin (P = 0.027). Of the azole derivatives, isavuconazole proved to be the most active agent (all strains with MICs < 1 μg/mL) (P = 0.05), followed by posaconazole, and voriconazole. Itraconazole showed the lowest activity (20.7% of strains MICs > 2 μg/mL) (P < 0.001). We observed a statistically significant correlation (P < 0.001) in terms of activity between pairs of agents as follows: isavuconazole–posaconazole (PC = 0.639), isavuconazole–itraconazole (PC = 0.648), voriconazole–posaconazole (PC = 0.786), voriconazole–itraconazole (PC = 0.810), itraconazole–posaconazole (PC = 0.654) and caspofungin–micafungin (PC = 0.410).

Conclusions: Amphotericin B showed lower antifungal activity against A. nidulans than the other agents. In contrast, terbinafine, the echinocandins, and the new triazoles had good antifungal activity against A. nidulans. Positive correlations between the MICs of the four azoles and between the MICs of micafungin and caspofungin were found. J. Guinea (FIS CM05/00171) and M. Torres-Narbona (CM08/00277) are contracted by FIS.

**P1299 In vitro combination of amphotericin B or posaconazole with cyclosporine A against zygomycetes**

E. Dannaoui*, P. Schwarz, O. Lortholary (Paris, FR)

Objectives: to evaluate the in vitro interaction between antifungals and cyclosporine A, a calcineurin inhibitor, against Zygomycetes.

Methods: ten isolates of Zygomycetes (3 Rhizopus oryzae, 2 Mycocollus corymbifer (formerly Absidia corymbifera), 2 Mucor circinelloides, 2 Rhizopus microsporus var. rhizopodiformis, and 1 Rhizomucor pusillus) were used. RPMI agar plates containing predefined concentrations of cyclosporine A were inoculated with a spore suspension by swabbing the agar surface. The concentration of cyclosporine A incorporated in the agar plates was two-fold lower than the Minimum Inhibitory Concentrations (MIC) for isolates inhibited by <8 mg/l, or 16 mg/l for isolates which were not inhibited by 8 mg/l of cyclosporine A.

**P1297 Is Aspergillus nidulans susceptible to all antifungal agents? In vitro activity of an updated panel of antifungal agents against 63 clinical isolates**


Background: Aspergillus nidulans is an infrequent but potential cause of invasive aspergillosis. However, its in vitro antifungal susceptibility profile has been poorly evaluated. We studied the antifungal susceptibility of an updated panel of 8 antifungal drugs against 63 clinical strains of A. nidulans collected in our hospital since 1997.

Methods: The strains were from respiratory samples (n = 55), superficial samples (n = 5), and other samples (n = 3) and were identified according to morphological characteristics. Antifungal susceptibility to amphotericin B, terbinafine, caspofungin, micafungin, itraconazole, voriconazole, posaconazole and isavuconazole was obtained following the CLSI M38-A procedure and the results analyzed using the t test and Pearson correlation coefficient (PC).

Results: The antifungal susceptibility (MIC50, MIC90, geometric mean and ranges, in μg/mL) was as follows: amphotericin B (4/4.3/3.1−8), terbinafine (0.062/0.125/0.09/0.031−1), caspofungin (0.125/0.25/0.38/0.062−8), micafungin (0.062/0.062/0.065/0.062−0.125), itraconazole (1/2/1.036/0.25−4), voriconazole (0.5/0.2/0.61/0.062−2), posaconazole (0.5/0.1/0.54/0.025−1) and isavuconazole (0.25/1/0.46/0.062−1). Amphotericin B showed the lowest activity (52.3% of strains with MICs > 4 μg/mL) (P < 0.001). In contrast, terbinafine showed good activity (98.4% of strains with MICs ≤ 0.25 μg/mL). Micafungin showed higher activity than caspofungin (P = 0.027). Of the azole derivatives, isavuconazole proved to be the most active agent (all strains with MICs < 1 μg/mL) (P = 0.05), followed by posaconazole, and voriconazole. Itraconazole showed the lowest activity (20.7% of strains MICs > 2 μg/mL) (P < 0.001). We observed a statistically significant correlation (P < 0.001) in terms of activity between pairs of agents as follows: isavuconazole–posaconazole (PC = 0.639), isavuconazole–itraconazole (PC = 0.648), voriconazole–posaconazole (PC = 0.786), voriconazole–itraconazole (PC = 0.810), itraconazole–posaconazole (PC = 0.654) and caspofungin–micafungin (PC = 0.410).

Conclusions: Amphotericin B showed lower antifungal activity against A. nidulans than the other agents. In contrast, terbinafine, the echinocandins, and the new triazoles had good antifungal activity against A. nidulans. Positive correlations between the MICs of the four azoles and between the MICs of micafungin and caspofungin were found. J. Guinea (FIS CM05/00171) and M. Torres-Narbona (CM08/00277) are contracted by FIS.
Amphotericin B or posaconazole Etest strips were applied on the agar surface and MIC of antifungals were determined after 24 h of incubation at 35°C.

**Results:** A decrease of ≥2 dilutions of the MIC of the antifungal, suggesting synergy, was observed for 50% of the isolates for the combination of amphotericin B with cyclosporin A, and for 60% of the isolates for the combination of posaconazole with cyclosporin A. Antagonism was never observed.

**Conclusion:** For most isolates of zygomycetes, the calcineurin inhibitor cyclosporine A enhanced the antifungal activity of either amphotericin B or posaconazole. These preliminary results should be confirmed in vivo in an animal model. Positive interactions between antifungals and immunosuppressive drugs may have clinical implications for treatment of solid organ transplant patients with zygomycosis.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (µg/1) of the Antifungal</th>
<th>Concentration (µg/1)</th>
<th>Cytotoxicity of the drugs in combination with CyA</th>
<th>MIC (µg/1) of the drugs in combination with CyA</th>
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<tbody>
<tr>
<td>R. oryzae IP 1640.83</td>
<td>ND</td>
<td>1</td>
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<td>ND</td>
</tr>
<tr>
<td>R. oryzae IP 4.77</td>
<td>≥2</td>
<td>≥2</td>
<td>0.5</td>
<td>≥2</td>
</tr>
<tr>
<td>R. oryzae CNR 013.98</td>
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<td>≥2</td>
<td>0.9</td>
<td>0.5</td>
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<tr>
<td>R. oryzae IP 1105</td>
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<td>≥2</td>
<td>1.5</td>
<td>0.5</td>
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<tr>
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<td>2</td>
<td>0.25</td>
<td>1.5</td>
</tr>
<tr>
<td>M. circinelloides CNR 05.154</td>
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<td>≥2</td>
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<tr>
<td>M. circinelloides CNR 05.371</td>
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<td>≥2</td>
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<tr>
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<td>A. corymbifera IP 1280.81</td>
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<td>A. corymbifera IP 1279.7</td>
<td>ND</td>
<td>0.5</td>
<td>16</td>
<td>ND</td>
</tr>
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</table>

**Cya:** cyclosporin A; **AMB:** amphotericin B; **PSZ:** posaconazole; **ND:** Not done.

**P1300 Molecular mechanisms ofazole resistance in *Candida glabrata* clinical isolates from Slovakia**

S. Borecka, N. Berila, V. Dzugasoua, J. Bohnansky, J. Subik* (Bratislava, SK)

**Objectives:** The aim of this study was to investigate the molecular mechanisms involved in a decreased susceptibility to azole antifungals in unmatch *C. glabrata* clinical isolates recovered from different patients treated in two university hospitals in Slovakia. The attention was also paid to cell surface hydrophobicity and in vitro biofilm formation associated with virulence of fungal pathogens.

**Methods:** Drug susceptibilities were determined by microbroth dilution method in 96-well plates according to the proposed CLSI (formerly NCCLS), M27-A standard guidelines and by E-test assay. PCR was carried out with a high-fidelity KOD Hot Star DNA polymerase (Sigma-Aldrich, St. Louis, USA). The DNA sequence was determined with the ABI Prism 3100 DNA sequencer. The quantitative real time PCR was carried out in the 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, USA). Cell surface hydrophobicity was measured by the water-octane two phases assay. Biofilm formation was quantified biochemically as XTT reduction.

**Results:** Molecular mechanisms of resistance toazole antifungals were investigated in ten unmatch *C. glabrata* clinical isolates. Overexpression of the CgPDR1 gene encoding the main multidrug resistance transcription factor and its target genes CgCdr1 and CgCdr2 coding for drug efflux transporters was observed in six fluconazole resistant isolates. Sequence analysis of the PCR amplified DNA fragments of their CgPDR1 gene identified the L347F and H576Y amino acid substitutions in five clinical isolates, and showed no upregulation of drug efflux transporter genes due to novel gain-of-function mutations in gene encoding CgPdr1p transcription factor.

**P1301 Parallel-resistance of azoles and echinocandines and their cross-resistance to fluconazole and amphotericin B in clinical yeast isolates**

A. Schmalreck, K. Becker, W. Fegeler* (Münch, Munster, DE)

**Objectives:** To evaluate the parallel-resistance (PR) of azoles (AZs) (fluconazole, FL; itraconazole, IT; voriconazole, VO; posaconazole, PO) and echinocandines (ECs) (caspofungin, CA; anidulafungin, AN) and their cross-resistance (CR) to fluconazole (FC) and amphotericin B (AMB), the minimum inhibitory concentrations (MICs) of 1,355 clinical yeast isolates (CYIs) were analyzed by susceptibility pattern analysis (SPA).

**Methods:** Testing of CYIs was performed by Etest® strips on modified RPMI agar with 2% glucose and 0.5 mg methylene blue/L. All antifungal agents (AFAs) were tested in parallel from the same inoculum (10⁵ cfu/mL). SPAs of the individual susceptibility results for each AFA in each isolate were performed and the results were aligned to individual susceptibility patterns (SPPs).

**Results:** The CYIs comprised 9 genera: Candida (1266/93.4%, comprising 749 C. albicans, 358 C. glabrata, 88 C. tropicalis, 49 C. parapsilosis and 22 isolates of other species), Clavispora (11/0.8%, Magnu- siomyces (8/0.6%, Filobasidiella (2/0.2%), Issatchenka (29/2.0%), Kluyveromyces (15/1.1%), Pichia (10/0.7%), Saccharomyces (13/0.9%), and Trichosporon (1/0.1%). The percentage of resistant isolates differed considerably depending on the AFA and on the MIC-reading time (24h vs. 48h): FC, 8.6 vs. 11; AM, 0.6 vs. 4.9; FL, 5.9 vs. 19.4; IT, 27.7 vs. 33.5; VO, 2.2 vs. 6.5; PO, 15.1 vs. 27.6; CA, 1.6 vs. 3.5; AN, 3.1 vs. 4.8. The species-specific resistance rates (RRs) demonstrated large variations showing RRs for ECs of C. dubliniensis, C. parapsilosis, and Pichia from 10% to 85% and RRs for AZs of C. glabrata, Issatchenka, and Pichia from 20% to 90%. CR of the AZs and ECs tested to FC and AM was not found in any isolate, even after 48 h of incubation. According to 24h vs. 48 h incubation, 28 vs. 79 isolates showed complete PR to AZs (C. glabrata, 25 vs. 53; C. albicans, 2 vs. 23) and 776 (57.3%) vs. 681 (50.3%) strains exhibited susceptibility to all antifungals tested. PR with ECs was recorded in 18 (1.3%) vs. 29 (2.1%) of the strains.

**Conclusion:** The MIC differences and the one-sided PR of the azoles underline the importance of analysing the routine-results with different comparative analysing tools. Susceptibility determination of AFA was found to be strongly species- and endpoint-reading dependent. Consequently, species-specific breakpoints for these agents should be established in order to achieve reliable in vitro results and qualified therapy recommendations.

**P1302 Varying interactions between voriconazole and amphotericin B against Candida spp. in an in vitro kinetic model**

A. Lignell*, E. Löwdin, O. Cars, J. Sjölin (Uppsala, SE)

**Objectives:** An antagonistic effect of voriconazole (VRC) on the fungicidal activity of sequential doses of amphotericin B (AMB) against Candida albicans (CA) has previously been demonstrated. It may be speculated that this is an effect only seen in strains that are susceptible to VRC. The aim of the present study was to investigate, in an in vitro kinetic model, whether a similar inhibitory effect was seen between VRC and AMB against CA, and known mechanisms of resistance against VRC, and against Candida glabrata (CG) and Candida krusei (CK) with varying susceptibility to VRC.

**Methods:** Four strains of CA and two strains each of CG and CK were tested. To a starting inoculum of 10⁶ CFU/mL of yeast in sterile RPMI 1640, antifungal agents (VRC: 5.0 mg/L, AMB: 2.5 mg/L) were added and placed at 35 degrees Celsius. Antifungal containing medium was eliminated from the culture vessel and replaced by fresh medium with a peristaltic pump at a flow-rate adjusted to obtain a 6-h half-life of VRC. A computer controlled dosing pump compensated for the 7-h half-life of AMB. Repeated sampling for viable counts was made. Fungal killing
was defined as the difference in log10 CFU/mL before and 6 h after administration of AMB. Fungicidal activity was defined as a reduction in CFU/mL of ≥3 log10.

Results: Against CA, the regimens with AMB and simultaneous AMB + VRC resulted in a fungicidal activity within 1.5 h. When CA was exposed to VRC for 24 h before administration of AMB, fungal killing were reduced in three out of four strains. Fungal killing (standard error in brackets) were 2.0 (0.3), 2.6 (0.4), 4.8 (1.1), and 0.3 (0.1) log10 CFU/mL (MIC: 2, 8, 8, and 256 µg/mL, mechanism of resistance: efflux pumps CDR1, CDR1, MDR1 and uncharacterised, respectively).

Against CG and CK, AMB alone resulted in fungicidal activity within 1.5–6 h. Simultaneous AMB + VRC decelerated the fungicidal killing rate. When CG and CK were exposed to VRC for 24 h before AMB, fungal killing were reduced in all strains. The numbers were 1.3 (0.1), 0.7 (0.3), 0.4 (0.2) and 1.7 (0.4) log10 CFU/mL (MIC: 0.5, 4 (CG), 0.5, 4 (CK) µg/mL, respectively).

Conclusion: An inhibition of the fungicidal activity of AMB by VRC against CA was seen despite reduced VRC susceptibility. The interaction seems more dependent on the type of resistance mechanism rather than the MIC-level. Furthermore, a reduction of the fungicidal activity of AMB by VRC was demonstrated against strains of CG and CK.

**P1303**

**Assessment of the in vitro pharmacodynamic activity of echinocandins against Candida parapsilosis**

C. Big, J. Vazquez* (Ann Arbor, Detroit, US)

Objectives: Echinocandins have become commonly used antifungals in the management of invasive candidiasis despite the fact that the reported MICs of *C. parapsilosis* (Cp) for echinocandins are much higher than the MICs of other *Candida* spp. The main objective was to evaluate the in-vitro pharmacodynamic activity of echinocandins against Cp.

Methods: 12 Cp isolates recovered from the bloodstream of different patients were evaluated. MIC/MFC and Time Kill Assays (TKA) were done for Cfgn, Anidulafungin (Afgn), Fluconazole (FLZ), Voriconazole (VCZ), and AmB, using CLSI methodology.

Results: TKA of Cfgn, Afgn, VCZ, and AmB using Cfgn-susceptible and -resistant strains revealed no fungicidal activity at 0.5 and 2× the MIC for Cfgn and Afgn, when compared to control strains of *C. albicans* (Ca) and *C. glabrata* (Cg). However, at 4× and 8× the MIC, both Cfgn and Afgn demonstrated fungicidal activity against Ca and Cg at 0.5 and 2×MIC at the 6 and 48 hours time points. In contrast, both Cfgn and Afgn demonstrated fungicidal activity against Ca and Cg at 0.5 and 2×MIC at the 6 and 48 hours time points, respectively.

Conclusions: Cfgn and Afgn demonstrated concentration-dependent activity, which was either fungistatic or fungicidal, depending on the *Candida* spp. evaluated and the concentration used. Excellent fungicidal activity was achieved with Cfgn and Afgn at 4× and 8× MIC against different strains of Ca. However, when compared to the activity displayed against Ca and Cg, the efficacy was inferior. The echinocandins as used today may not be the best options in the management of candidaemia due to *Candida parapsilosis*.

**P1304**

**Antifungal susceptibility of *Candida parapsilosis* bloodstream isolates during the last decade**

A. Spiliopoulou*, S. Vamvakopoulos, L. Pikoula, C. Bartzawali, E.D. Anastassiou, M. Christofidou (Patras, GR)

Objectives: Non albicans *Candida* species have emerged as important nosocomial pathogens as the epidemiology of candidaemia has changed over the years. Among 245 *Candida* bloodstream isolates, antifungal susceptibility data of 34 cases attributed to *Candida parapsilosis* is presented.

Methods: A ten year surveillance of bloodstream infection (BSI) caused by *C. parapsilosis* was carried out in the Microbiology Laboratory of University Hospital of Patras. Gram stain of material from positive blood culture vials (Bact/Alert, Organon Teknika) was examined microscopically for yeasts, whereas, another portion was subcultured on Sabouraud dextrose agar (SDA, Difco, USA). All SDA plates were incubated at 35°C for 72 h and yeast-like colonies were isolated. Germ tubes formation is indicative of *C. albicans*, whereas, germ tubes negative *Candida* isolates were identified by API 20 AUX (BioMerieux), an assimilation test of carbohydrates. Susceptibility testing (MIC) to antifungal agents was carried out by E-Test (AB Biodisk). MIC was evaluated according to CLSI criteria for amphotericin B (AP), 5-flucytocine (FC), fluconazole (FL), ketoconazole (KE), itraconazole (IT), voriconazole (VO), posaconazole (POS) and caspofungin (CS).

Results: *C. parapsilosis* was the most frequent non-albicans *Candida* species (14%), isolated from BSI. Fifteen candidaemia cases (44%) due to *C. parapsilosis* were identified in Neonatal Intensive Care Unit (NICU), thirteen cases (38%) in Internal Medicine Ward, Haematology-Oncology Unit and Transplantation Center, five cases in Surgical Wards (General Surgery Units and Orthopedics), whereas, only one case was identified in adult ICU. Susceptibility data showed that all *C. parapsilosis* isolates were sensitive to AP, FC, VO, POS and IT. In terms of resistant to other azoles, four isolates were resistant to FL, two to KE and one to IT.

Conclusions: Almost half (44%) of *C. parapsilosis* isolates were identified in NICU, where *C. parapsilosis* is the most common (25%) *Candida* non albicans causing BSI.

In Surgical Wards and in adult ICU, the incidence of *C. parapsilosis* BSI is rather low. Parenteral alimentation and the widespread use of central venous catheters in low birth-weight infants have been linked to *C. parapsilosis* infections. Among azoles, the highest degree of resistance was observed to FL (12%) and ke (6%), whereas POS and VO were active against all isolates tested. No resistance was found to AP, FC and CS.

**P1305**

**In vitro susceptibility of clinical isolates of Saccharomyces cerevisiae**


Background: The incidence of opportunistic invasive fungal infections has increased dramatically in recent years. *Saccharomyces cerevisiae*, a yeast used in food industry and as a probiotic, is increasingly reported as aetiological agent of human infections, and considered as a possible emerging pathogen. We have reviewed the antifungal susceptibility profile of a collection of clinical isolates of *S. cerevisiae* in order to give any insight on the management of this emerging infection.

Methods: A total of 57 isolates received in our institution over a period of 11 years (1997 to 2007) was evaluated. The isolates were identified by routine morphological and physiological tests. MICs of amphotericin B (AMB), fluconazole (FLC), voriconazole (VRC), ravuconazole (RVC), posaconazole (POS), caspofungin (CAS), micafungin (MCF) and anidulafungin (AND) were determined according to the recommendations proposed by the European Committee on Antifungal Susceptibility Testing for fermentative yeast (EUCAST-definitive document).

Results: Most of the 57 strains were isolated from vaginal exudates (15/57, 26.3%), oropharyngeal exudates (11/57, 19.3%), blood (8/57, 14.0%), and biopsy specimens (7/57, 12.3%). A slight increase in the number of *S. cerevisiae* strains received was detected in the last years (1.31% to 3.36% from 1997 to 2007). The majority of the strains (54/55, 98.1%) were considered susceptible in vitro to AMB (tentative MIC breakpoint MIC ≤1 mg/L). However different rates of susceptibility to azoles have been reported: High MICs of FLC (>4 mg/L) were detected in 35% of the strains. Whereas, elevated MICs of IT, VRC, RVC and POS (MICs >1 mg/L) were detected in 18.6%, 7.8%, 2.4% and 5.3% of the strains respectively. In addition, echinocandins demonstrated great activity against most of the strains. (GM for CAS, MCF and AND were respectively 0.36, 0.04 and 0.08 mg/L).

Conclusions: This species seems to be an emerging pathogen and should not be dismissed as a non-pathogenic microorganism when recovered from clinical sources. AMB, new azoles and echinocandins showed good in vitro activity against this fungus.
Antifungal susceptibility of 205 Candida yeast isolated during systemic candidiasis. Comparison of the Vitek 2 system with E-Test and CLSI broth microdilution methods

N. Bourgeois*, L. Dehandchooevercker, S. Bertout, P. Rispaill, L. Lachaud (Nîmes, FR)

Objective: The objective of this study was to evaluate a new automated antifungal susceptibility test system (AST-YS01 Vitek 2 cards, Biomerieux) (VK2). It was compared with the Etest procedure and the CLSI reference broth microdilution method. For this purpose, 205 clinical isolates of Candida spp were tested with amphoterin B, fluconazole and voriconazole.

Methods: The CLSI and the Etests MICs were determined at 24 and 48 hours of incubation at 35°C. The amphoterin B MIC was determined as the lowest concentration inhibiting any growth. The azole MICs were read as the lowest concentrations producing 50% (CLSI) and 80% (Etest) reduction of growth. The MICs with the VK2 cards were noticed spectrophotometrically after variable incubation time, depending on the growth control. All the methods were validated each time with quality control strains. Interpretative breakpoints available for fluconazole and voriconazole were used to calculate categorical agreement percentages.

Results: Essential agreement (EA) between the VK2 and CLSI method was excellent. For VK2 and etest, EA depended on Candida species and molecule tested. The interpretation for susceptibility to fluconazole at 48 hours end point with the three methods was fully concordant in all the Candida albicans isolates: 98.8% were susceptibles (S), and 1 isolate resistant (R). For Candida glabrata, only 17.86% of the isolates were given the same interpretation for fluconazole with the three methods (7S, 2R, 1SDD). Categorical agreement was 78.6% between the VK2 and CLSI method, while only 23.2% between VK2 and Etest method. A cross-resistance for voriconazole was noticed in 12.5%, 10.6% and 51.8% with VK2, CLSI and Etest methods respectively. All the Candida parapsilosis (n = 22), Candida tropicalis (n = 17), and Candida lusitaniae (n = 2) were found S for fluconazole and voriconazole with the three methods. It is noteworthy that none of the Candida krusei was found R to fluconazole with VK2 system, and that the MIC corresponded to a susceptible value for 6/14 isolates. Nevertheless, the VK2 expert does correct the interpretation to resistant. For amphoterin B, a MIC ≤ 1 was globally found for 201 (VK2), 190 (CLSI), and 202 (etest) isolates. One Candida glabrata isolate had a MIC > 2 in the three methods.

Conclusion: The AST-YS01 Vitek 2 cards system (Biomerieux) is a reliable and practical automated antifungal susceptibility test.

Comparison of the Vitek ASTM-YS01 card with CLSI broth microdilution reference method for testing fluconazole and voriconazole against C. neoformans

A.I. Aller*, C. Castro, A. Romero, M.T. González, R. Claro, E. Martin-Mazuelos (Seville, ES)

Objective: The purpose of this study was to compare the results obtained by the reference microdilution method (MDM) with those obtained with the commercial method AST-YS01 card (YS01) for antifungal susceptibility testing of C. neoformans to fluconazole (FZ) and voriconazole (VZ)

Materials and Methods: We have studied retrospectively 89 clinical isolates of C. neoformans. The susceptibility to two azoles was performed by the MDM according to the CLSI guidelines (M27-A2 document) modified by Ghannoum et al (J. Clin. Microbiol. 1992; 30:2881–86). The MICs were read at 48–72 h. of incubation. The YS01 was performed according to the manufacturer's instructions. The inoculum suspensions of C. neoformans isolates matched the turbidity of n 2 McFarland standard. The MICs were read at 24–48 h. of incubation.

The interpretative criteria for VZ were those published by the CLSI (Document M27-A3 and M27-S3). For FZ the isolates with MIC > 16 mg/L were considered resistant and the isolates with MIC < 8 mg/L were considered susceptible, as suggested previously by Aller A.I. et al (Antimicrob Agents Chemother 2000; 44: 1544–48). C. krusei ATCC 6258, C. parapsilosis ATCC 22019, C. neoformans 90112 and C. neoformans 90113 were included as control strains.

Results: For FZ 23 isolates of C. neoformans (25.8%) failed to grow in the Vitek 2 system at 24 h. and they needed to be read after 24 h. of incubation (mean time of 28 h. with a range from 25 to 32 h.). The overall essential agreement (EA) between the two methods was of 97.7% to FZ. For VZ only 12 isolates grew well in the YS01 (13.5%), making it impossible to obtain results. The categorical agreement between both methods for FZ is summarized in table 1. YS01 was not able to identify the C. neoformans isolates resistant to FZ (2/4, 50%).

Conclusions: 1. These data suggest the potential value of YS01 for determining the MICs of FZ to susceptible C. neoformans isolates.
2. YS01 failed to recognize the FZ resistant C. neoformans isolates.
3. According to our study, YS01 is not a reliable method for susceptibility testing of C. neoformans to VZ.
4. Further studies including a higher number of strains resistant to both antifungal agents are necessary.
The 7-isolates of C. glabrata were tested in-house [Flucnazole MIC 8 mg/L] and at Mycology Regional Laboratory, Wythenshawe hospital [MIC – Micafungin (<0.015 mg/L); Caspofungin (0.5 mg/L); Voriconazole (0.125–0.5 mg/L); Fluconazole (8 mg/L) and Amphotericin (0.06 mg/L)].

**Discussion:** Uncertain correlation between higher MIC and successful clinical outcome with *C. parapsilosis* is reported. Therapeutic failure with caspofungin in a susceptible *C. glabrata* strain is of concern. Micafungin, newest of echinocandins, has lower MICs against *C. glabrata* and offered clinical success.

**Results:** We identified 7 caspofungin-resistant *Candida* spp isolates (1%) obtained from 7 patients (median age 56 years, range 17–87; 57% were males). All patients had haematological malignancy and 4 had received prior corticosteroid treatment. Seven patients were liver transplant recipients, 5 had haematologic malignancies and 2 had AIDS. Five out of 12 patients were infected by *Candida non-albicans* strains. All *Candida* isolates were sensitive to caspofungin according to the CLSI breakpoints (MIC < 2 mcg/ml). Mean Cmax, Cmin and AUC were respectively 8.36±2.18 mg/L, 1.98±0.87 mg/L and 80.45±28.84 h*mcg/ml*. Mean plasma half-life was 15.5±4.7 hours and the mean plasma volume of distribution at steady state was 8.63±1.98 L/kg. Considering a MIC = 2 mcg/ml, mean Cmax/MIC and AUC/MIC resulted respectively 4.18 and 40.2.

**Conclusion:** Caspofungin PK values measured in our patients were similar to those reported in healthy men, confirming a prolonged half-life and a high volume of distribution. Moreover, our data highlight that the Cmax/MIC and AUC/MIC ratios may be acceptable even with MIC values equal to the higher value of in vitro sensitivity. Such PK parameters are clearly higher with clinical isolates of *Candida* with MIC lower than 2 mcg/ml. The role of TDM of caspofungin in the human clinical setting needs further studies.

**Materials and Methods:** We reviewed the in vitro susceptibilities (CLSI method) of 650 *Candida* species associated with candidiasis or non-candidem invasive candidiasis episodes in 582 hospitalised cancer pts at MDACC (2005–2008). We retrospectively reviewed the characteristics of the candidiasis episodes caused by CAS-R *Candida* species (MIC > 2).

**Results:** We identified 7 caspofungin-resistant *Candida* spp isolates (1%) obtained from 7 patients (median age 56 years, range 17–87; 57% were males). All patients had haematological malignancy and 4 had received prior corticosteroid treatment. Seven patients were liver transplant recipients, 5 had haematologic malignancies and 2 had AIDS. Five out of 12 patients were infected by *Candida non-albicans* strains. All *Candida* isolates were sensitive to caspofungin according to the CLSI breakpoints (MIC < 2 mcg/ml). Mean Cmax, Cmin and AUC were respectively 8.36±2.18 mg/L, 1.98±0.87 mg/L and 80.45±28.84 h*mcg/ml*. Mean plasma half-life was 15.5±4.7 hours and the mean plasma volume of distribution at steady state was 8.63±1.98 L/kg. Considering a MIC = 2 mcg/ml, mean Cmax/MIC and AUC/MIC resulted respectively 4.18 and 40.2.

**Conclusion:** Caspofungin PK values measured in our patients were similar to those reported in healthy men, confirming a prolonged half-life and a high volume of distribution. Moreover, our data highlight that the Cmax/MIC and AUC/MIC ratios may be acceptable even with MIC values equal to the higher value of in vitro sensitivity. Such PK parameters are clearly higher with clinical isolates of *Candida* with MIC lower than 2 mcg/ml. The role of TDM of caspofungin in the human clinical setting needs further studies.
A new real-time PCR assay for the direct detection of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium

G. Clarebout *, C. Bordet, C. Calie, O. Prudillon, L. Monfort, P. Sednaoui (Marnes-la-Coquette, Paris, FR)

**Objective:** Bio-Rad has developed a new Real Time PCR assay for the direct detection of Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG) and Mycoplasma genitalium (MG) from DNA extracts of First Void Urine (FVU) or vaginal, urethral, cervical, and rectal swabs samples resuspended into a transport medium. This study was conducted in order to (1) evaluate the clinical performances of this new assay versus Roche COBAS TaqMan CT and Amplicor NG tests; (2) prove the interest of MG screening.

**Methods:** The study took place at Institute Alfred Fournier (IAF) Paris, France, a STI-specialised medical centre. The reference assays were Roche Cobas TaqMan48 for CT, Roche Cobas Amplicor and culture for NG and an in-house PCR for MG. For the reference and Bio-Rad assays, DNA was extracted with a manual extraction method, provided for the kit.

**Results:** 276 samples were tested with the Bio-Rad and the reference assay, including 137 FVU and 139 swab specimens (4 urethral, 114 cervical, 20 rectal and 1 throat). CT: 51 samples were positive with both techniques. The clinical sensitivity and specificity of the Bio-Rad assay were 100% versus the reference test. NG: 36 of 36 positive samples were in agreement with the three techniques. 3 Bio-Rad False Positive (FP) samples were detected Roche Cobas TaqMan48 for CT, Roche Cobas Amplicor and culture for MG and an in-house PCR for MG. For the reference and Bio-Rad assays, DNA was extracted with a manual extraction method, provided in the kit.

**Conclusion:** The Bio-Rad test presents performances similar to Roche tests for the detection of CT and NG. Moreover, this study points out the importance of routine testing for MG, a pathogen too often left untreated because undetected, particularly in patients co-infected with CT or NG.

**Evaluation of a commercial assay for fast and efficient detection of Chlamydia trachomatis-DNA in clinical specimens with a rapid PCR-based dipstick assay**

M. Weizenegger *, J. Bartel (Heidelberg, DE)

**Objectives:** The purpose of this study was to evaluate the new molecular dipstick assay GenoQuick® CT (Hain LifeScience, Nehren, Germany) and a fast and cost effective DNA isolation system (GenoLyse®) for the direct and specific detection of Chlamydia trachomatis in urine and cervical swabs.

**Methods:** 88 urines and 69 swabs (VIDAS Chlamydia, BioMérieux, Nürtingen, Germany) were analysed with the GenoLyse®-DNA-isolation kit and the GenoQuick® CT assay. Results were compared to data received from the COBAS TaqMan® CT (Roche Diagnostics, Mannheim, Germany). Swabs were washed out in 1 ml of sterile saline. Either 500 µl urine or saline was transferred to a tube and centrifuged. The supernatant was discarded and the pellet resuspended in 100 µl of lysis reactant. After a 5 min heating step in boiling water 100 µl of neutralisation reagent was added. PCR was performed in a final volume of 50 µl containing 5 µl of the DNA-solution and 1 U HotStar-Taq (Qiagen, Hilden, Germany). 10 µl of the PCR-amplification mix was analysed with a PCR-dipstick in 100 µl running buffer in a deep well micro tier plate. The stick contains 3 lines for wild type detection, amplification control and run control and were evaluated after 10 min by eye.

**Results:** The QG CT assay showed for urine samples sensitivity, specificity, positive predictive and a negative predictive value of 100%, 98.5, 95.2 and 100% and for cervical swabs of 100%, 96.3%, 75.0%, and 100% compared to the COBAS® TaqMan® CT results.

**Conclusion:** The GenoQuick CT dipstick assay for the direct detection of Chlamydia trachomatis is proved to be rapid, sensitive and specific. The turnaround time is approximately 4 hours. In opposite to other assays no cost intensive equipment is needed.

**Chlamydia trachomatis detected in a Croatian national public health institute between 2004–2008**

A. Babić-Ercég *, S. Ljubin-Siernuk, G. Vojnović, N. Bauk (Zagreb, HR)

**Objectives:** Chlamydia trachomatis is a pathogen of global public health significance. Its infection is one of the most important sexually transmitted diseases. The diagnosis relies entirely on laboratory techniques. Chlamydial research has been guided and determined by developments in diagnostic technology.

**Materials and Methods:** Sample collection: Cervical and urethral swabs were transported in 2-SP transport medium, and first-voided urine specimens, sperm specimens and expressed prostatic secretions (EPS) in the sterile containers. Two methods were used:

1. Isolation on McCoy cells (ECACC, UK) with detection by group specific monoclonal antibodies (Bio-Rad).
2. Nucleic acid amplification tests (NAATs)
   a. PCR Cobas Amlicor CT/NG method (Roche Diagnostics)
   b. Real time PCR (TaqMan technology, ABI7500, Applied Biosystems) using probe and primers retrieved from the database at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/blast). DNA extraction was performed with Amplicor CT/NG specimen preparation kit (Roche) for swabs and QIAamp Blood mini Kit (Qiagen) for other specimens.

**Results:** In a four year period, total of 41902 specimens from outpatients (36615 female, 4477 male) were examined by cell culture method (N = 27919) and by one of the NAATs (N = 13173). There were 33173 cervical swabs, 5924 urethral swabs (3107 female, 2817 male), 541 urine samples (335 female, 206 male), 640 sperm specimens and 814 EPS. The overall prevalence of C. trachomatis infection was 1.59% (667/41902). There were 511/33173 (1.5%) positive cervical swabs, 84/2817 (2.9%) male urethral swabs, 35/3107 (1.1%) female urethral swabs, 10/206 (4.8%) male urine samples, 4/335 (1.2%) female urine samples, 5/640 (0.6%) sperm specimens and 19/814 (2.3%) EPS. There was no infection in NAATs.

**Conclusion:** Our results in a four year period show low prevalence of C. trachomatis infection. There is lower prevalence in female population...
than in male. It is also lower when using cell culture instead of PCR. There is an absolute need for testing sperm samples by PCR because of its toxicity to culture cells.

**P1312**

A molecular assay detecting quinolone resistance in *Neisseria gonorrhoeae*: treatment with ciprofloxacin can still be an option

R. Nijhuis*, B. Slotboom, T. van Zwet (Velp, NL)

**Objectives:** In the Netherlands, resistance of ciprofloxacin in *Neisseria gonorrhoeae* (quinolone resistant *Neisseria gonorrhoeae* (QRNG)) has increased rapidly in recent years to about 30%. As a consequence guidelines for the initial treatment of this infection have been altered. In the majority of cases, where no susceptibility pattern is available, intramuscular cefotaxim is now regarded the first option.

To make a more specific approach possible for the individual patient, we wanted to implement a polymerase chain reaction (PCR) detecting these QRNG.

**Methods:** Using a Real Time PCR (JCM 2004:42; 3281–3283) targeting the intact gyrA and parC quinolone resistance-determining regions (QRDRs), we aimed to detect whether the *Neisseria gonorrhoeae* is susceptible, intermediate or resistant to ciprofloxacin. This method is a good model for resistance detection using nucleic acid amplification tests since the QRNG resistance mechanisms are based on stepwise accumulation of point mutations. In this PCR, a signal for gyrA as well as for parC should indicate susceptibility, a signal for gyrA or parC an intermediate susceptible strain, whereas no signal should signify resistance.

Performance of this assay was tested using 63 isolates with a known resistance. The total of 26 susceptible, 6 intermediate and 31 resistant isolates were evaluated. In case of discrepant results, E-testing was performed (S: MIC <0.06; I: MIC 0.12–0.5; R: MIC ≥1.0).

**Results:** Of the 26 susceptible isolates, 24 gave a signal with both gyrA and the parC genes. In the remaining two only parC gene was detected. E-testing of these two isolates revealed one susceptible and one intermediate strain. In the 6 intermediate isolates and the 31 resistant isolates, a 100% correlation with the traditional culture method was seen.

**Conclusion:** This PCR real time assay has the potential to differentiate between susceptible and non-susceptible isolates of *Neisseria gonorrhoeae* without using culture methods. This informs the clinician whether treatment with ciprofloxacin instead of a third generation cephalosporin is still an option.

**P1313**

Metagenomic assessment of sputum microbiota from patients with chronic pulmonary diseases

V. Friaza, C. de la Horra, G. Ayala, L. Máz, J. Dapena, R. Canton, E. Calderón, R. del Campo* (Seville, Madrid, ES)

**Objective:** To identify the main features of microbiota associated with cystic fibrosis (CF) and bronchiectasis, two models of chronic lung diseases, using a non-culture microbiological approach.

**Methods:** Sputum samples (one per patient) were collected from 15 CF-patients and from 15 patients with non-CF bronchiectasis. All patients presented a stable clinical status without acute exacerbations. Total DNA from each sputum sample was obtained manually using a phenol/chloroform protocol and diluted up to 50 ng/µl. PCR-DGGE technique was performed in all samples using universal primers for Bacterial Domain based on 16S rRNA conserved regions. Amplicons were separated in vertical electrophoresis polyacrylamide gels (8%) at 60°C; with a urea-formamide denaturating gel gradient of 30–45%. Gels were separated in vertical electrophoresis polyacrylamide gels (8%) at 60°C; with a urea-formamide denaturating gel gradient of 30–45%.

**Results:** All CF-patient samples presented a marked band of *Pseudomonas aeruginosa*, which was undetectable in those patients with bronchiectasis. On the other hand, several band patterns were common to both groups. As expected, different species corresponded to bacteria habitually found in sputum samples (*Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Moraxella* spp., *Actinomyces* odontolyticus.) although several sequences corresponded to uncultured bacterium related with *Streptococcus*, *Actinobacterium* or *Neisseria* groups. Interestingly, environmental organisms such as *Pseudomonas* *borkenhavia*, *Ochrobactrum anthropi*, *Rothia amarae*, *Rothia mucilaginosa*, *Phychococcus dokdonensis*, or *Arthrobaacter* spp. were detected.

**Conclusion:** Metagenomic tools are useful to identify the microbiota present in patients with chronic pulmonary diseases. Moreover, we were able to detect uncultured and environmental bacteria in sputa from CF and non-CF bronchiectasis patients that have not been previously described in this type of samples.

**P1314**

Use of novel electrochemical labels in a highly sensitive, multiplex detection method for use in a rapid point-of-care molecular assay

M. Green*, D. Pearce, A. Dixon, T. Jenkins, C. Frost (Trowbridge, Bath, UK)

**Objectives:** To demonstrate the advantages of novel ferrocene-based electrochemical labels used in a highly sensitive multiplex assay for *Chlamydia trachomatis*, compatible with a rapid point-of-care diagnostic platform. To show how a range of electrochemical labels with different oxidation potentials can be used in a multiplex assay for *C. trachomatis*, *Neisseria gonorrhoeae* and an internal control.

**Methods:** *Chlamydia trachomatis* specific PCR primer set was used to asymmetrically amplify target DNA extracted from Elementary Bodies (EB). A single-stranded DNA probe was synthesised with the electrochemical label linked to the 5’ base via the terminal phosphate group. This probe was hybridised to the *C. trachomatis* ampiclon, then incubated in the presence of a double-stranded DNA specific exonuclease (T7 exonuclease). The released label was measured by performing a voltammetric scan on the samples using screen printed carbon electrodes. The *C. trachomatis* assay (above) was run in a multiplex assay with *Neisseria gonorrhoeae* and an internal control, using specific primers and probes for each target. An electrochemical label, with a unique oxidation potential, was coupled to each probe, these were then hybridised and incubated with the exonuclease in the multiplex mix. A single voltammetric scan was performed using screen printed carbon electrodes.

**Results:** All CF-patient samples presented a marked band of *Pseudomonas aeruginosa*, which was undetectable in those patients with bronchiectasis. On the other hand, several band patterns were common to both groups. As expected, different species corresponded to bacteria habitually found in sputum samples (*Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Moraxella* spp., *Actinomyces* odontolyticus.) although several sequences corresponded to uncultured bacterium related with *Streptococcus*, *Actinobacterium* or *Neisseria* groups. Interestingly, environmental organisms such as *Pseudomonas* *borkenhavia*, *Ochrobactrum anthropi*, *Rothia amarae*, *Rothia mucilaginosa*, *Phychococcus dokdonensis*, or *Arthrobaacter* spp. were detected.

**Conclusion:** Metagenomic tools are useful to identify the microbiota present in patients with chronic pulmonary diseases. Moreover, we were able to detect uncultured and environmental bacteria in sputa from CF and non-CF bronchiectasis patients that have not been previously described in this type of samples.

Table 1. Results for PCR with electrochemical end-point detection of a dilution series of *Chlamydia trachomatis* Elementary Bodies and DNA from common cross-reactants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oxidation potential (mV)</th>
<th>Mean electrochemical measurement (nA)</th>
<th>Standard deviation %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 EB*</td>
<td>192</td>
<td>216.33</td>
<td>11.02</td>
</tr>
<tr>
<td>1000 EB*</td>
<td>198</td>
<td>109.47</td>
<td>25.17</td>
</tr>
<tr>
<td>100 EB*</td>
<td>198</td>
<td>65.07</td>
<td>4.66</td>
</tr>
<tr>
<td>1 EB*</td>
<td>201</td>
<td>54.97</td>
<td>25.29</td>
</tr>
</tbody>
</table>

No DNA/EB Control – 0 – 0 –
E. coli – 0 – 0 –
Strep. agaractiae – 0 – 0 –
Candida albicans – 0 – 0 –
* S. gonorrhoeae – 0 – 0 –
E. faecalis – 0 – 0 –
S. aureus – 0 – 0 –
Lactobacillus sp. – 0 – 0 –
* T. vaginalis – 0 – 0 –
Human – 0 – 0 –

*EB = *Chlamydia trachomatis* Elementary Bodies; †Higher %CV due to sampling at low level dilutions

**Results:** The *C. trachomatis* molecular assay has been demonstrated to show a limit of detection down to a single EB. Negative controls (no EBs and non-target DNA), showed no voltammetric measurement...
above baseline level (see data). The assay was performed on all 15 C. trachomatis serovars and demonstrated 100% specificity against a panel of 90 possible cross-reactants. The multiplex electrochemical assay using C. trachomatis, N. gonorrhoeae and internal control provided discrete voltammetric signals at the 3 oxidation potentials for the electrochemical labels, when measured using a single voltammetric scan. Removal of an individual target from the multiplex assay, resulted in removal of the voltammetric peak at the associated oxidation potential.

Conclusions: The electrochemical detection method demonstrates a high level of sensitivity in a C. trachomatis assay and has shown to be capable of providing a multiplex platform for a diagnostic test panel. The methods and materials used in these tests were directly compatible with a rapid point-of-care platform.

**P1318 MALDI-TOF MS based identification of Staphylococcus hominis using the BioTyperTM software**

J.K.M. Knobloch*, F. Machoca, W Sollbach, P Petrus (Lubeck, DE; Prague, CZ)

**Objective:** Staphylococcus hominis strains are one of the major staphylococcal species inhabiting the skin of humans. Staphylococcus hominis is subdivided in the subsp. hominis and novobiosepticus. Staphylococcus hominis subsp. novobiosepticus is a multiresistant pathogen occurring with increasing frequency in human clinical material like aetiological agent of nosocomial infections. Most commercial identification systems are not able to differentiate these subspecies. We evaluated the performance of the BioTyperTM software for MALDI-TOF MS based bacterial identification for the differentiation of the S. hominis subspecies.

**Methods:** 20 isolates of S. hominis subsp. hominis and 22 isolates of S. hominis subsp. novobiosepticus from different years and origins were analysed by MALDI-TOF mass spectroscopy using direct smear and Ethanol-formic acid extraction (EFAE) for sample preparation. The resulting mass spectra were automatically analyzed using the BioTyperTM database. Currently, the database has 6 entrances for S. hominis (3 subsp. hominis, 1 subsp. novobiosepticus, and 2 type strains without subspecies designation).

**Results:** All 42 strains were correctly identified as S. hominis on the species level with high score values (>2.3) independent of the preparation method. In the majority of strains the highest similarity was calculated to one of the S. hominis reference strains without subsp. designation (32 by direct smear and 40 by EFAE). A correct classification of the subsp. was only observed in 4 strains by direct smear and 2 strains by EFAE. Using direct smear as preparation method a bias in the identification towards S. hominis subsp. hominis was observed independent of the respective subspecies. Using EFAE as preparation method a bias towards S. hominis subsp. novobiosepticus become evident (second best similarity for 36/42 strains).

**Conclusions:** The BioTyperTM system for MALDI-TOF based bacterial identification is highly accurate in the species identification of S. hominis even in a simple direct smear preparation on the target plate. Subsp. identified by the BioTyperTM database should currently not be reported without further biochemical analysis. More Database entrances and further bioinformatic processing are required to differentiate the S. hominis subsp. by the BioTyperTM software.

**P1319 Mutation induction characteristics and parameters of antibiotic stress**

A.T. Wong*, M.L. Chin, W.C. Chan, M.L. Ling (Hong Kong, HK)

**Objectives:** The term “Adaptive Mutation” describes a cellular mechanism in bacteria that generates genetic changes in non-growing population under stress. The difficulty to differentiate whether newly emerged mutants are due to selection or induction by stress results in controversies over its existence. This study aimed at determining whether target-specific adaptive mutation played a role in conferring antibiotic resistance to bacteria. Four major aspects of antibiotic resistance gene mutations were studied – drug specificity in resistance induction, mutation site specificity, rate of mutation formation and physiological stages in which mutations preferentially developed.

**Methods:** The study approach involved phenotypic screening and analysis of several categories of resistant mutants of a model organism Escherichia coli, which were collected under different physiological conditions with or without antibiotic stress, as well as subsequent determination of drug susceptibility profiles and sequencing of specific genes. Four antibiotics of different classes (ampicillin, ciprofloxacin, rifampicin and gentamicin) were used in cross induction and screening experiments. The number and phenotypes of resistant isolates that emerged at various physiological stages, under short and long term exposure to different levels of antibiotic stresses, were used to depict the mutation induction characteristics of specific antibiotic stress.

**Results:** In an attempt to assess mutation induction specificity of antibiotics, the cellular process governing the emergence of antibiotic resistant cells was found insensitive to drug induction. Mutation development was highly dependent on bacterial physiological stages. Rifampicin and gentamicin resistant isolates existed before antibiotic induction and selection, however, such mutants were not cross-resistant to other antibiotics, indicating presence of site-specific mutant subgroups among the spontaneous mutants in various bacterial populations. Analysis of mutation patterns of resistance genes in isolates collected from various induction conditions provided further information on the specificity of the mutations which emerged before or after the antibiotic induction.

**Conclusion:** Our data suggest the presence of specific physiological processes that regulate the production, nature and size of antibiotic-resistant mutant populations. Such processes appear to be sensitive to starvation rather than antibiotic stress.

**P1320 Mycobacterium tuberculosis cpsA is a master regulator in adaptation to the macrophage phagosome**

M. Alzohairy*, J.E. Graham (Barayrah, SA)

Mycobacterium tuberculosis maintains a relatively large genetic repertoire for adaptation to a variety of different environments encountered during typical respiratory infections. Adaptation to these in vivo niches is critical in maintain and sustaining infection. An environment of central relevance in all infections, and among the first encountered, is that within the phagosomes of human macrophages. Global analyses of bacterial RNAs produced in response to phagocytosis produced a list of candidates including that predicted to encode the transcriptional regulator CpsA. We investigated the contribution of cpsA regulated gene expression to M. tuberculosis pathogenesis and identified a large regulon consisting of about 30 genes whose transcript levels were increased by increased cpsA expression. These included transcriptional regulators sigE, sigB, Rv0195, and indicating a regulatory cascade initiated by cpsA expression following phagocytosis. Bacterial mutants unable to express cpsA were impaired in intracellular growth and this defect was complemented by restoration of cpsA. We provide the initial characterisation of a key transactional activator mutating a global regulatory response allowing M. tuberculosis to colonise its normal ecological niches in the macrophages phagosome.

**P1321 Evaluation of MTB Q Alert kit for detection of Mycobacterium tuberculosis directly on patient samples**

A.C. Mendes*, S.J. Fernandez, L.C. Ferreira, M. Pereira, H. Ramos, J.M. Cabeda (Porto, PT)

**Objectives:** Laboratory diagnosis of active tuberculosis is still very time consuming, since culture takes weeks to months for positivity, and the molecular biology assays generally used for this purpose are very labour intensive. Thus the clinical need for a fast diagnosis of tuberculosis would benefit from the use of Real-time-PCR techniques that are generally very fast, automated and extremely sensitive and
Development and application of a multiplex polymerase chain reaction assay for rapid differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis

J.D. Colmenero*, M.I. Queipo-Ortuño, P. Bermudez, A. Plata, P. Morata (Málaga, ES)

Objectives: The aim of this study was to develop a multiplex real-time PCR (M RT-PCR) assay to simultaneously detect Brucella spp and Mycobacterium tuberculosis complex DNA and analyze its yield in the rapid differential diagnosis between extrapulmonary tuberculosis and certain focal complications of brucellosis.

Methods: A broad panel of Brucella and Mycobacterium strains and forty-five consecutive non-blood clinical specimens from 25 patients with different focal complications of brucellosis and 18 patients with extrapulmonary tuberculosis were studied by M RT-PCR assay. Control samples were obtained from 26 patients with other disorders initially involving a differential diagnosis with extrapulmonary tuberculosis or brucellosis. For the detection of Brucella spp, a 207 bp fragment from the conserved region of the gene which encodes an immunogenic membrane protein of 31 kDa of B abortus (BCSP31) specific to the Brucella genus and present in all its biovars was amplified using the primers B1 and B2. Primers M1 and M3 amplifying a sequence of 164 bp based on the intergenic region of the genes coding for a mycobacterial two-component system SenX3-RegX3 were used for the identification of Mycobacterium tuberculosis complex. To confirm the identities of the amplified fragments, sequence analysis was carried out. Sensitivity, specificity, positive and negative predictive values, accuracy, likelihood ratios (LR) and 95% confidence intervals (CI) of M RT-PCR were calculated.

Results: The detection limit of the M RT-PCR was 2 genomes per reaction for both pathogens and the intra- and inter-assay coefficients of variation were 0.44% and 0.93% for Brucella and 0.58% and 1.12% for Mycobacterium. M RT-PCR correctly identified 42 of the 45 samples from patients with tuberculosis or brucellosis and was negative in all the controls. Thus, the overall sensitivity, specificity, PPV and NPV values of the M RT PCR assay were 93.3%, 100%, 100% and 89.7%, respectively, with an accuracy of 95.8% (95% CI, 91.1%-100%).

Conclusions: Since M RT-PCR is highly reproducible and more rapid and sensitive than conventional microbiological tests, this technique could be a promising and practical approach for the differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis.
Results: In each isolate, the chromosomal location of the Hie cap locus was identical to that previously described for *H. influenzae* serotype f strain 700222 and associated with the same flanking genes (sodC at the 3′ end and HI1637 at the 5′ end). No sequences reminiscent of the IS1016 element were found to flank the locus. The Hie cap locus included 14 ORFs organised in three distinct functional regions (I, II and III). Both regions I and III showed high sequence identity to the previously described corresponding regions from Hib and Hif. Eight new ORFs (named ecs1 to ecs8) were identified in the Hie capsule-specific region II. All isolates were found to contain only one copy of the Hie cap locus.

Conclusion: Chromosomal location of the Hie cap locus is conserved among our isolates. Although Hie strains belong to the major phylogenetic division 1 (majority of serotypes a, b strains and all serotypes c, d, e and strains), the Hie cap locus appeared to be located in the same chromosomal site as that of serotype f strains. The Hie cap locus is not flanked by direct repeats of IS1016 and, accordingly, it is not amplified. Genes within the locus are organised in three distinct regions as other group II capsule biosynthetic clusters.

**P1325** Global comparison of recA gene sequences from clinical and environmental *Vibrio cholerae* strains isolated in Iran

A. Dashtbani *, B. Bakhshi, M. Pourshafie (Tehran, IR)

Objectives: *Vibrio cholerae* is a natural inhabitant of the aquatic environment. Pathogenic strains of *V. cholerae* can cause cholera disease which is one of the major concerns in the health system. Recently, it was hypothesized that some pathogenic strains evolved from environmental nonpathogenic strains. We aimed to determine the genetic relatedness and pattern of nucleotide sequence variation among clinical and environmental *V. cholerae* strains in Iran based on recA gene analysis. We also compared our sequences with that of strains from different countries available online in the database.

Methods: We amplified internal fragment of recA gene of 10 environmental and 12 clinical strains of *Vibrio cholera* isolated from surface waters and hospitalised patients from Iran during 2004–2008. PCR products, sized 1100 bp, were subjected to sequencing. DNA sequence data were analyzed with MEGA4 program. Sequences of recA gene of 203 strains were obtained from NCBI database. Alignment of all the mentioned sequences were performed. We found 530 nucleotide in common. The gene tree was constructed by UPyGMA method.

Results: From the 530 nucleotide (nt), 395 nt (74%) were conserved among all the global strains. We found 135 variable sites. Of which, 25 nt (4.71%) and 110 nt (20.75%) were singleton and phylogenetically informative, respectively. One of our environmental isolates was identical to clinical ones based on recA sequence. We observed the most variation among the environmental isolates (59 nt) whereas the clinical isolates showed at most 5nt difference with each other. Three of our environmental isolates had unique sequences but the others were clustered with strains from some other countries. Most of the clinical isolates grouped together forming the largest cluster containing 71 members which included 8 of our clinical isolates. The other two Iranian clinical isolates formed a separate cluster.

Conclusion: DNA-based typing methods enables researchers to compare their results with other countries online and draw conclusions about the evolution of pathogenic bacteria. Our findings suggest that analysis of recA gene sequences can be used as a reliable tool for global investigations of *V. cholerae* population structure. In future, we analyze some other housekeeping genes for drawing more robust conclusion about the genetic variation in *Vibrio cholerae* population.

**P1326** Characterisation of diversity in wbeT region of the O-antigen biosynthesis gene cluster in Ogawa and Inaba serotypes of *Vibrio cholerae*

A. Sharifi*, B. Bakhshi, M. Hashemi, M.R. Pourshafie (Tehran, IR)

Objectives: *Vibrio cholerae* serotype O1 Inaba and Ogawa have been associated with widespread cholera outbreaks which occur annually in Iran. An approximately 22 kb gene cluster is responsible for O1 antigen biosynthesis. Serotype switching occurs within this cluster in the genome of *V. cholerae* O1. It has been identified that product of wbeT region in the cluster is a transferase enzyme and is responsible for the expression of the B determinant, which is Ogawa-specific.

We determined to investigate any deletion in the wbeT region of the O1 antigen biosynthesis gene cluster responsible for serogroup conversion in the *V. cholerae* O1 strains.

Methods: Thirty *V. cholerae* isolates of clinical origin, isolated and subjected to analysis. Serogrouping of isolates performed using O1 polyvalent Ogawa and Inaba monospecific antisera. PCR assay performed to analyze any deletion in the wbeT region from the beginning up to end. Restriction fragment length polymorphism (RFLP), done by using EcoRI restriction enzyme to digest the products and determine any deletion or point mutation within the restriction sites of the enzyme. Complete sequence analysis of wbeT region performed to assess any variation in this region responsible for serogroup conversion in Inaba isolates.

Results: The results of serogrouping showed that 66% isolates were Inaba and 34% were Ogawa. An approximately 800 bp product obtained for all of the isolates under study. RFLP analysis revealed no diversity in the restriction sites of the enzymes nor any deletion in the fragments obtained from the assay. Sequence analysis of the amplified fragments performed by Clustal software, determined substitution of C for T in 295 in all Inaba strains. In addition to the unique mutation which mentioned above, the strain 62013 (*V. cholerae* O1, Classic, Ogawa) which used as positive control in all PCR assays, showed another substitution which was A for C in 310. The recent mutation only reported in the strain 62013.

Conclusion: Results of this study indicate that no obvious deletion has occurred in the wbeT region of Inaba strains in comparison to Ogawa. Complete sequence analysis determined a substitution of Pro for Ser. Another mutation which occurred in the strain 62013, led to substitution of Lys for Gin. Since all of the strains under study were El Tor biotype and 62013 was the only strain which was Classic, it seems that the nucleotide in 310 in Classic biotypes can be an appropriate place for point mutations.

**P1327** Improved detection of bacterial gastro-intestinal pathogens using molecular screening

R.F. de Boer *, A. Ott, B.J. Kezsityi, A.M.D. Kooistra-Smid (Groningen, NL)

Objectives: Traditional methods to detect gastro-intestinal (GI) pathogens are slow, and/or lack sensitivity. Molecular detection of GI pathogens has proven to be rapid, sensitive and feasible in a routine microbiology setting (Schuurman, T. et al., JCM (45):3692–3700). Since December 2006 a molecular screening approach for detection of bacterial GI pathogens has been implemented in our microbiology laboratory. This study describes the improved detection frequency of bacterial enteric pathogens using multiplex real-time PCR (mPCR) in 2007.

Methods: During 2007, 10189 stool samples were sent to our laboratory, and analysed with conventional culture (39.9%) or screened for the presence of bacterial enteric pathogens with mPCR (61.1%). Using mPCR, samples were screened for *Salmonella enterica* (SE), *Campylobacter jejuni* (CI), *Shiga* toxin-producing *Escherichia coli* (STE), and *Shigella* spp./enteroinvasive *E. coli* (SH/EIEC). All PCR positive stool samples as well as samples that demonstrated PCR inhibition were additionally tested with conventional culture (mPCR guided culture). Detection frequencies of the molecular approach were compared with those found using conventional culture for SE, *Campylobacter* spp., STEC O157, and SH.

Results: Using the molecular approach, the detection frequencies were 2.5% for SE, 9.2% for CI, 2.0% for STEC, and 1.5% for SH/EIEC. A total of 133 (84%), 435 (77%), 9 (7.5%) and 19 (20%) mPCR positive samples could be confirmed with culture for SE, CI, STEC, and *Shigella* spp. respectively. Detection frequencies using conventional culture were 3.0% for SE, 5.8% for *Campylobacter* spp., 0.6% for STEC O157, and
Development and evaluation of internal amplification controls for real-time duplex PCR to detect Campylobacter coli and C. jejuni

L.P. Randall*, A. Vidal, J. Rodgers, F. Clifton-Hadley (New Haw, UK)

Objectives: A common problem of both conventional and real time PCRs is failure of DNA amplification due to the presence of inhibitory substances in the samples, particularly when using DNA from faecal samples. In view of this, our aim was to develop/evaluate Internal Amplification Controls (IACs) for use with an existing duplex real time PCR for Campylobacter coli and C. jejuni.

Methods: The Campylobacter PCR detected the ceuE gene of C. coli and the mapA gene in C. jejuni. Both competitive and non-competitive IACs were developed/evaluated. The competitive approach involved a DNA fragment (60 bp) of the coding region of the viral fish haemorrhagic septicaemia virus, flanked by the mapA PCR primers, whilst the non-competitive approach utilised an extra set of universal 16S primers. Both IAC PCR types were evaluated using pure cultures of C. coli or C. jejuni and suitable negative control cultures as well as with chicken caecal DNA extracts and diluted faecal supernatants, to study inhibitory effect of faeces on the PCR. Duplicate naturally-infected and artificially-spiked chicken caecal samples were prepared with Campylobacter (coli or jejuni) present at c. 106, 104 and 102 cfu/g. DNA was extracted from these samples and from a further 20 "field" faecal samples using the “ExtraMaster Fecal DNA extraction kit”.

Results: Both the competitive (fish virus) and non-competitive (16S) IACs had the potential to reduce the sensitivity of the PCR, although the competitive (fish virus) was more sensitive to faecal inhibitors than the C. coli or C. jejuni components of the PCR, making them both suitable to detect inhibition which could lead to false negatives.

Conclusion: Both IACs were shown to work in the duplex real time PCR, but the competitive IAC was chosen as the preferred option, as the signal for this IAC was not compromised by positive signals for C. coli or C. jejuni. Both of the IAC’s were more sensitive to faecal inhibitors than the C. coli or C. jejuni components of the PCR, making them both suitable to detect inhibition which could lead to false negatives.

Whole genome sequencing of pathogenic and non-pathogenic treponemes: comparison of syphilis and yaws strains with Treponema paraluiscuniculi

D Smaja*, M. Zobaníková, D. Čejků, P. Matejková, M. Strouhal, G.M. Weinstock (Brno, CZ; St. Louis, US)

Objectives: Treponema pallidum subsp. pallidum (TPA) is the causative agent of syphilis, T. pallidum subsp. pertenue (TPE) causes endemic treponematosis yaws, and T. paraluiscuniculi is not infectious to humans but causes venereal syphilitis in rabbits. TPE and TPA treponemes are morphologically indistinguishable and their genomes show nearly 99% identity to the genome of T. paraluiscuniculi. To identify these subtle genetic differences, whole genome sequencing is required.

Methods: The genomes of TPE strain SamoA D and T. paraluiscuniculi strain Cuniculi A were sequenced using several whole-genome sequencing methods including comparative genome sequencing, pyrosequencing, Solexa sequencing, and dideoxyterminator sequencing. Obtained whole genome sequences were compared to the published sequence of TPA strain Nichols.

Results: The genome size of TPA Nichols (1,138,006 bp), TPE SamoA D (1,139,299 bp) and T. paraluiscuniculi Cuniculi A (1,133,391 bp) and the overall gene order is similar in all investigated genomes. More than 50% of Nichols and SamoA D genes encode sequentially identical proteins whereas only 15% of those genes were found in the Cuniculi A and SamoA D genomes. Major sequence changes including framshifts were found in 30 genes of the SamoA D genome and in 88 genes of the Cuniculi A genome when compared to the Nichols genome.

Conclusions: Our results showed that the TPE SamoA D sequence is more closely related to the reference Nichols genome than the Cuniculi A sequence. Observed genetic changes are responsible for different pathogenicity and host specificity of syphilis and yaws treponemes compared to T. paraluiscuniculi. This work was supported by the grants from the Internal Grant Agency of the Ministry of Health of the Czech Republic (NR/3967-4/2006) and from the Grant Agency of the Czech Republic (310/07/0321).

Study on the prevalence of tick-borne pathogens in the Lublin region (eastern Poland)

A. Wójcik-Fatla, E. Cisak*, J. Chmielewska-Badora, J. Zwolinski (Lublin, PL)

Objectives: The aim of the study was to evaluate infection rates of Borrelia burgdorferi, Anaplasma phagocytophilum and tick-borne encephalitis virus in ticks collected in from different provinces of the Lublin region.

Methods: One thousand eight hundred thirteen Ixodes ricinus ticks (67% males, males and nymphs) collected from the interior of the Lublin region were examined for the presence of Borrelia burgdorferi sensu lato DNA and Anaplasma phagocytophilum DNA by PCR method. Fifty seven I. ricinus ticks from three provinces of the Lublin region were tested in pools for tick borne encephalitis virus (TBEV) by mice inoculation and cell culture. Dermacentor reticulatus ticks collected from the Poleski National Park, situated in the eastern part of the Lublin upland (Włodawa district) were examined individually for TBEV by nested RT-PCR method according to Schrader and Siss after a total RNA extraction by RNeasy Mini kit (Qiagen, USA).

Results: The infection rate of B. burgdorferi DNA in I. ricinus fluctuated from 1.7% to 10.9% and the mean infection rate amounted to 5.4%. The percentage of I. ricinus ticks infected with A. phagocytophilum was within the range of 0.8%-8.3% (mean 5.9%). The minimum infection rate of TBEV in I. ricinus ticks collected from Radzion Podlaski district was estimated as 4.2% and in the total area of the Lublin region was...
Towards a comprehensive proteome mapping of the extracellular metalloprotease complex of *Actinobaculum schaalii*.

J. Grossmann, S. Barkow-Oesterreicher, B. Roschitzki, L. Eberl, P. Carranza, I. Hartmann, A. Lehner, R. Stephan, P. Gehrig

1. Borrelia burgdorferi, Anaplasma phagocytophilum and tick-borne encephalitis virus circulate in forest environment of the Lublin region and indicate a risk for residents as well as for visitors.
2. The obtained results indicated a necessity for further study on TBEV in the whole Lublin region.

Acknowledgement: This study was partly supported by the Polish Ministry of Science and Higher Education, Grant No. N N404 029435.

**P1331** Towards a comprehensive proteome mapping of the opportunistic pathogen *Chromobacter turicensis* 3032

P. Carranza, I. Hartmann, A. Lehnre, R. Stephan, P. Gehrig, J. Grossmann, S. Barkow-Oesterreicher, B. Roschitzki, L. Eberl, K. Riedel* (Zurich, CH)

Members of the genus *Chromobacter* are notorious opportunistic pathogens associated with contaminated milk powder formulas for neonates; however, our knowledge about virulence mechanisms or natural reservoirs is still scarce. The proteome of *Chromobacter turicensis* 3032, which has recently caused 2 deaths in the Children Hospital of Zurich, was mapped aiming on a better understanding of physiology and putative pathogenic traits of this clinical isolate. Our analyses of extracellular, surface-associated and whole-cell proteins by two complementary proteomics approaches, one-dimensional gel-electrophoresis coupled to liquid chromatography and electrospray ionisation tandem mass spectrometry and two-dimensional liquid chromatography coupled to matrix-assisted laser desorption/ionisation time-of-flight tandem mass spectrometry, lead to the identification of 853 proteins corresponding to a remarkably 20% of the theoretically expressed protein complement of *C. turicensis*. The majority of the identified proteins are involved in central metabolic pathways, translation, protein folding and stability; moreover, we detected several potential virulence factors, expression of which was confirmed by phenotypic assays: a macrophage infectivity potentiator involved in *C. turicensis* persistence in host cells, a superoxide dismutase protecting the pathogen against reactive oxygen species, and an enterobactin-receptor protein for the uptake of siderophore bound iron. Most interestingly, an insecticidal chitinase and metalloprotease but no casein protease of *C. turicensis* 3032 originates from an environmental habitat rather than a milk-processing production site.

**P1332** Extracellular metalloprotease complex of *Proteus mirabilis*: a bio-informatic study

L. Michelm*, J. Fracasso, A.P. Delamare, S. Echeverrigaray (Caxias do Sul, BR)

Objectives: Metalloproteases, particularly mirabylisin (ZapA), are considered important virulence factors of *Proteus mirabilis*. Mirabylisin is active against a broad range of proteins including IgA and IgG. In this study we report a bioinformatic study of the metalloprotease complex of *P. mirabilis*, as well as a structural analysis of mirabylisin.

Methods: The nucleotide and aminoacid sequences of ZapA gene and Zap operon of *P. mirabilis*, as well as the orthologous sequences present in other bacterial species were obtained from NCBI. Promoters prediction was carried out using BPROM and PPP, and rho-independent terminators and RNA secondary structure were analyzed using FindTerm and RNAfolder. The comparison of mirabylisin sequence with other proteins was carried out using BLASTp. Orthologous protein sequences from different genera were aligned using Clustal X, and a dendrogram was generated by the Mega 4.0.1 program using the Neighbour-joining algorithm. ZapA conserved domains were identified by the Conserved Domain Architecture Retrieval Tool (CDART) of NCBI. Sequence alignment was carried out using the ClustalX program and the results were edited in the BioEdit environment. The structural model of ZapA protein of *P. mirabilis* was determined using the Swiss-model Workspace. The 3-D protein model used to predict ZapA structure was the serralysin of *Serratia marcescens*. The stereochemical quality of the ZapA protein model was evaluated by Procheck and Ramachandran plot.

Results: The results showed the presence of: (1) five metalloprotease genes located in a contiguous region within bacterial genome; (2) two different structural and functional proteases in *P. mirabilis*, one represented by four highly similar Zn-metalloproteases with a long C-terminal Peptidase M10 calcium-binding domain, and a typical serralysin Zn-metalloprotease represented by mirabylisin (ZapA); (3) a sigma 28 promoter controlling the Zap operon transcription; and (4) a precocious rho-independent terminator within Zap operon.

Conclusion: The structure of mirabylisin exhibited the typical N-terminal ZnMc serralysin domain, characterised by an α-β structure with a HExGHxxGLxH motif, and the C-terminal peptidase M10 domain formed by nine β-rolls with calcium binding G-rich nonapeptides.

**P1333** Actinobaculum schaalii, a common cause of urine tract infections?

S. Bank*, J. Prag (Viborg, DK)

Objectives: *Actinobaculum schaalii* is a slow growing, CO2 demanding, trimethoprim and ciprofloxacin resistant Gram positive rod and has been reported as a cause of urine tract infections, occasionally with bacteraemia. Due to its slow growth, also under optimal conditions *A. schaalii* is often overgrown with faster growing commensal and pathogen bacteria and is therefore only found in monocultures in large quantities. Identification by morphology and biochemistry often takes weeks. Since most microbiological laboratories routinely culture urine overnight at 37°C in ambient air using Columbia blood agar and MacConkey agar *A. schaalii* is generally overlooked.

We developed a TaqMan real-time quantitative PCR assay targeting the gyrB gene to rapidly detect *A. schaalii*. The real-time PCR assay has been used to determine the presence and to get a hint to a better understanding of the clinical importance of *A. schaalii*. Methods: A universal primer set was used to sequence the gyrB gene from fourteen *A. schaalii* strains including a *A. schaalii* reference strain CCUG 27420. The sequences were used to develop a TaqMan real-time quantitative PCR assay targeting the gyrB gene of *A. schaalii*. The assay was then tested against 37 *A. schaalii* isolates as well as several genetically related and clinically relevant bacterial strains. Finally the assay was used to screen 150 clinical, consecutive urine samples from patients above 60 years of age.

Results: Of the 150 urine samples 22 were found to harbour a load of more than 10^5 *A. schaalii* CFU/ml and 11 with less than 10^5. Of the 33 samples where *A. schaalii* was detected there were 27 with 60 patients suffered from chronic UTI are often blindly treated with ciprofloxacin to which some patient respond well whereas others do not show improvements after prolonged treatment. If *A. schaalii* is the cause of infection other antibiotics are needed to cure the infection. The real-time PCR assay can be used for fast diagnosis which will lead to better antibiotic treatment and thereby faster recovery and lesser admission.

**P1334** Comparison of alpha-amylase enzyme production between immobilised and submerged cells by native Bacillus licheniformis G

F. Mahmoudnia*, A. Pourbabaee, N. Ghaemi (Fars, IR)

Background: The possibilities of producing alpha-amylase with immobilised *Bacillus* cells have been investigated. This enzyme are widely used industrially for starch liquefaction.
The most frequently used immobilisation method is entrapment in gel matrices. The submerged productions of alpha-amylase using synthetic media have been reported by many workers.

**Objective:**
- To choose optimal conditions for immobilisation of growing cells of *Bacillus licheniformis* G in alginate.
- To evaluate alpha-amylase production by the immobilised cells in Batch fermentations.

**Methods:**
- To cultivation bacteria in preculture medium. (This biomass was used both for immobilisation and in experiments with free cells).
- To immobilisation of bacteria cells in Ca-alginate gel. (microencapsulation method)
- To carry out Batch fermentations with immobilised cells and free cells.
- alpha-amylase assay according to the method of Bernfeld by 3.5-dinitro salicylic acid reagent.

**Results:** The optimal immobilisation parameters (gel concentration, initial cell quantity, biomass age, Bead size and solidification prolongation) were determined.

The immobilisation procedure was most effective at a gel concentration of 3% using cells from a 12 h culture. The optimal initial cell quantity was found to be 2.6% in Ca-alginate gel with bead size of 5.0 mm and solidified for 24 h in 2.5% Calcium Chloride solution.

An enzyme yield of 20 U/ml culture medium was reached in Batch fermentation with immobilised cells. In contrast, the enzyme yield with immobilised cells was observed.

**Conclusion:** Higher gel concentration the rate of substrate mass transfer and the enzyme yield decreased.

Solidification of alginate improved the enzyme yield, presumably owing to decrease of the release from the beads. Amylase biosynthesis is also dependent on bead size, but in the case of initial cell loading in alginate, enzyme yield decreased at high initial cell density.

**Bacterial DNA in Egyptian patients with cirrhosis and culture-negative non-neutrocytic ascites: a marker of bacterial translocation and a prognostic indicator**

*N. Fam*, A. Ghali, A. El-Sayed, M. Shemis, M. Saber (Cairo, EG)

Bacterial translocation (BT) from intestinal wall to blood and other extra-intestinal sites is considered the key step in the pathogenesis of spontaneous bacterial peritonitis (SBP) in liver cirrhosis. The translocation of bacterial products as endotoxin and bacterial DNA, and the consequences of such translocations are under investigation.

**Objectives:** to study the presence of bacterial DNA and its possible role as a marker of BT in patients with advanced cirrhosis and culture-negative non-neutrocytic ascites (CNNA). The clinical significance of bacterial DNA as a possible prognostic marker in these patients was also evaluated.

**Methods:** 69 patients with cirrhosis and CNNA were included in the study. Bacterial DNA was detected in blood and ascitic fluid (AF) samples using polymerase chain reaction (PCR) for 16S ribosomal RNA. The corresponding bacterial DNA was identified by nucleotide sequencing of the purified PCR products by ABI 3130XL automated genetic analyzer (Applied Biosystem, USA). Results showed that bacterial DNA was detected in ascitic fluid and/or blood samples in 34.7% of patients (24/69). It was simultaneously found in both blood and ascitic fluid samples in 11 patients, in ascitic fluid samples only in another 11 patients and in blood samples only in 2 patients.

Nucleotide sequencing was performed for the DNA simultaneously found in blood and AF sand the similarity between the sequences found in both samples was >97% indicating single clonal origin. Nucleotide sequencing identified *Escherichia coli* as the main bacterial species detected in 70.8% of samples. *Klebsiella pneumoniae* was detected in 16.6% and *Staphylococcus aureus* in 12.5%. Follow-up of patients for 2 months showed a significantly higher mortality rate and progression to hepatorenal syndrome among patients with bacterial DNA in blood and/or AF compared to those without (p < 0.01, < 0.001).

**Conclusions:** These data represent the first detection of bacterial traces (DNA) in Egyptian patients with CNNA. It also provides molecular evidence of translocation of bacterial DNA that can serve as a prognostic marker predicting unfavourable outcome, so primary prophylaxis might be considered as a future perspective in such patients.

**Direct detection of Bordetella pertussis and B. parapertussis in clinical specimens by a rapid molecular dipstick assay**

*U. Eigner*, M. Hoffelder, A. Mahlke, A.M. Fahr (Heidelberg, DE)

**Objectives:** The purpose of this study was to evaluate the new molecular dipstick assay GenoQuick (GQ) Bordetella (Hain Lifescience, Nehren, Germany) for the direct and specific detection of *Bordetella pertussis* and *B. parapertussis*. Analytical specificity was calculated with 35 "non-Bordetella"-isolates of different culture collections (ATTCC, DSMZ and others). For the evaluation of the direct detection in clinical swab specimens, the GQ Bordetella assay was compared to a Real-Time-PCR assay specific for *B. pertussis*. Two different extraction methods (Hain Q-Lys-Method (Hain Lifescience) and EasyMAG (BioM´erieux, N¨urtingen, Germany)) were used. 80 patient specimens (30 positive, 50 negative with the Real-Time PCR) were collected from routine samples. Discrepant results were retested with *B. pertussis* and *B. parapertussis-specific* PCR assays.

**Results:** With regard to analytical sensitivity a lower median detection limit of 10 CFU/ml was determined. All 35 "non-Bordetella" isolates were tested negative with the assay. For clinical specimens the GQ Bordetella assay showed a sensitivity, specificity, positive predictive and a negative predictive value of 100%, 96%, 93.8%, and 100% with regard to the extraction method with EasyMAG and 100%, 100%, 100%, 100% with the Q-Lys-method, respectively. One B. parapertussis containing specimen was only tested positive when DNA was isolated with the EasyMAG system.

Time-to-result for the direct detection of *B. pertussis* and *B. parapertussis* from clinical specimens is 2.5 h with the molecular dipstick assay (2.25 h for amplification and 10 minutes for detection). Ca. 15 min have to be added for the Q-Lys-DNA-isolation procedure.

**Conclusions:** The GenoQuick Bordetella dip stick assay proved to be a rapid, sensitive and specific tool for the direct detection of *Bordetella pertussis* and *B. parapertussis* in clinical specimens within 2.5 h to 3 h.

**Molecular typing – part 2**

**Limitations of tpi and bg sub-genotypes for characterisation of human Giardia duodenalis isolates**

*J. Bonhomme, L. Le Goff, V. Lemee, G. Gargala, J.J. Ballet, J.F. Rossignol, L. Vuvennez* (Rouen, Caen, FR; Stanford, US)

The intestinal protozoan *Giardia duodenalis* is a cosmopolitan parasite frequently involved in human parasite gastroenteritis with two genetically different *G. duodenalis* assemblages A and B. Little is known so far on the genotypes of *G. duodenalis* strains which are infectious to humans in France.

**Objectives:** The present characterisation of 19 French clinical strains was aimed at determining their genotype patterns and associations with clinical symptoms, and in vivo metronidazole resistance, respectively.

**Methods:** Faecal human samples were purified and analysed using PCR amplification and direct sequencing of 2 fragment genes, i.e. triose phosphate isomerase (TPI) and b-giardin (BG).

**Results:** Twelve isolates were identified as assemblage A, and 7 as assemblage B for the gene loci. High intra-assemblage genetic variability determined many subgenotypes with incomplete overlap.
at the 2 loci. Using TPI gene analysis, 10/12 isolates belonged to subgenotype A2, and 2/12 genotype A and all B isolates could not be subgenotyped. Using BG gene analysis, 6/12 isolates belonged to genotype A2, 6/12 to genotype A1, 5/7 to genotype B1, and 2/7 genotype B isolates could not be subgenotyped. In addition, several heterogeneous nucleotide positions (i.e., the presence of 2 nucleotides at the same position) were highlighted in the two assemblages A and B and for both gene sequences. No association was found between genotype and clinical symptoms or metronidazole resistance, respectively.

Conclusion: Data support consensual improvements in multilocus G. duodenalis sub-genotyping strategies to better understand molecular epidemiology of giardiasis and the zoonotic potential of this parasite.

**Molecular typing – part 2**

**[P1338]** Multilocus sequence typing of *Campylobacter jejuni* isolated from bovines, poultry and patients in Finland

A. de Haan*, R. Kießtö, M. Hakkinen, H. Rautelin, M.L. Hänninen (Helsinki, FI)

**Objectives:** *Campylobacter* is known to be the major cause of bacterial gastroenteritis worldwide. It has been shown to be present in a variety of animal sources, but chicken is thought to be the main source of human *Campylobacter* infections. A clear peak of *Campylobacter* infections is observed in the summer months.

Multilocus sequence typing (MLST) has previously been employed to characterise *Campylobacter* and was used to identify sequence types (STs) in our datasets. The main aim of the present study was to map the distribution of MLST types of *Campylobacter* across Finland, since data on this is scarce and will help to better understand the epidemiology and source attribution in Finland.

**Methods:** A total of 107 bovine strains were isolated in 2003. The study included 71 chicken isolates from 2006 and 94 chicken isolates from 2007 (June-October included) as well. The bovine and poultry isolates were representative of the distribution of *Campylobacter* in the whole of Finland.

Eighty-nine isolates from patients with domestically-acquired infections were collected from the Helsinki-Uusimaa area in 2006 (July-December included). A total of 361 strains were typed by MLST. Sequence types were assigned by using the pubmlst database (http://publmlst.org/campylobacter/). The population structure and source attribution will be analyzed by the chi-square test, STRUCTURE and BAPS.

**Results:** Up to date 75% of all isolates have been successfully characterised by MLST. Of these, 13% of the strains were found to have a novel ST. Less than 5% of the strains were either not typable or *Campylobacter* was not recovered after primary isolation. According to preliminary findings, ST-21 and ST-61 clonal complexes were most prevalent in bovines, while ST-45 and ST-283 clonal complexes were more common in poultry. In patients ST-22 clonal complex was more frequently found compared to both bovine and poultry. The results of comprehensive data analysis will be presented at the conference.

**Conclusions:** The number of novel sequence types found seems to be slightly higher compared to published data. Nevertheless, the distribution looks fairly the same compared to the rest of Europe while ST-677 seems to be more prevalent in Finland.

**Bacterial isolation and identification was carried out according to the standard bacteriological methods. Arbitrarily primed PCR (AP-PCR) used to study the genetic relatedness between the *V. cholerae* isolates.**

**Results:** Thirty-nine isolates of *V. cholerae* O1 were identified. All isolates belonged to serotype Inaba. AP-PCR could differentiate the isolates into five groups. AP-PCR cluster types 1 and 2 were the most prevalent groups, accounting for 36% and 41%, respectively, of *V. cholerae* isolates.

**Conclusion:** The most of *V. cholerae* O1 strains could be attributed to two predominant clusters including AP-PCR cluster types 1 and 2 accounting for more than 77% of isolates. In conclusion, a few epidemic clones were responsible for the apparently epidemic occurrence of cholera in provinces studied.

**[P1340]** Comparison of PFGE and MLVA in subtyping of Salmonella Typhimurium strains

T. Kauko*, K. Haukka, A. Sitonen (Helsinki, FI)

**Objectives:** In Finland, microbiological laboratories send the Salmonella strains isolated from humans to the SalmoNet Reference Laboratory of THL for further typing. All *S. Typhimurium* strains isolated from the patients with domestically-acquired infection, were genotyped by pulsed-field gel electrophoresis (PFGE) and multiple-locus variable-number tandem-repeat analysis (MLVA). The discriminatory power and epidemiological concordance of MLVA and PFGE were compared and the epidemiological relatedness of Finnish *S. Typhimurium* strains was established.

**Methods:** Finnish *S. Typhimurium* isolates (*N* = 63) of human origin from the year 2008 were analysed by PFGE and MLVA. The strains belonged to the definitive phage types (DTs) 1, 104, 41, NST, U277, 104B, U322, 99, 195 and 120. PFGE was performed according to standardised PulseNet Europe protocol using XbaI restriction enzyme. MLVA was performed as described [1] with following differences: forward primers of loci STTR3 and STTR5 were labeled with NED fluorescence colour, locus STTR10 was labeled with PET fluorescence marker, LIZ600 served as internal standard and sequencing was carried out by capillary electrophoresis using the 3730xl DNA Analyzer (Applied Biosystems).

**Results:** DT1 (*N* = 22) and DT104 (*N* = 11) were the most common *S. Typhimurium* phage types. Salmonella isolates of DT1 were divided into three MLVA and five PFGE profiles. 20 of the 22 DT1 isolates belonged to the MLVA cluster 02–12–00–00–03 where only three loci are present. The MLVA cluster 02–12–00–00–03 was divided by PFGE into three XbaI macrorestriction profiles differing by one or more bands. The most common PFGE profile among the DT1 isolates, called STYM1 (*N* = 16), could not be divided further by MLVA.

MLVA analysis of the 11 DT104 isolates yielded five MLVA profiles and three XbaI profiles. The most common MLVA cluster 2–12–12–7–3 (*N* = 4) was divided into two different PFGE profiles.

**Conclusion:** Concordance between PFGE and MLVA was generally good. MLVA method is less discriminatory than PFGE, but in few cases, especially in epidemiological surveillance of *S. Typhimurium* DT104 it is a valuable tool. However, most of the isolates of *S. Typhimurium* DT1 which is the most frequent phage type in domestic human infections, show limited discrimination by MLVA due to present of only three polymorphic loci.

**Reference(s)**

are a less frequent cause of infections in humans but still are important pathogens. Serotype determination of GBS is based on the capsular polysaccharides and serotype determination on a variety of surface-anchored an strain-variable proteins, including C beta (bca) C alpha (bac), Alp1 (alp1), Alp2 (alp2), Alp3 (alp3), Alp4 (alp4), R4 (R4/Rib), and the R3 protein. In this study we have tested a collection of clinical GAS isolates for the presence of these GBS protein genes.

Methods: A total of 88 GAS strains isolated from infectious disease cases during the period 2004 to 2006 were examined. GAS strain R28 was used as alp3 positive reference strain. Multiplex PCR (J Clin Microbiol 42: 1326, 2004) was used to detect the genes mentioned above and antibody-based methods (APMIS 107: 869, 1999) to test for R3 protein expression.

Results: Of 88 clinical GAS strains examined, 23/88 (26.1%) were alp3 PCR positive. Of these, 2/14 (14.2%) blood culture isolates, 15/34 (44.1%) skin and soft tissue strains, and 6/40 (15%) respiratory tract strains possessed alp3. The frequency of possession of alp3 by the skin isolates was significantly higher than by the blood or respiratory strains (P < 0.05). All isolates were PCR negative for the genes bac (C beta protein), bca (C alpha protein), alp1 (epsilon), alp2 (Alp2), alp4 (Alp4), and rib (R4/Rib). R3 protein expression was not detected. Antibody-based testing of several isolates showed results in agreement with PCR results.

Conclusion: Our results show that the gene alp3 which encodes Alp3 in GBS and R28 in GAS, occurs with a high frequency in clinical GAS strains from our geographical area, with particularly high frequency in strains causing skin and soft tissue infections. It is a possibility that Alp3, which probably is identical to the T28 antigen, plays an important role in colonisation and pathogenesis of GAS-induced skin and soft tissue infections. One possibility is that Alp3/R28 functions as an adhesin which mediates attachment of GAS to receptor molecules in human skin. The other GBS genes tested in this study may never occur in GAS isolates.

**P1343** Pili are a clonal property of *Streptococcus agalactiae*
E.R. Martins*, J. Melo-Cristino, M. Ramirez (Lisbon, PT)

**Objectives:** *Streptococcus agalactiae* (GBS) isolates express protective antigens recognized as major components of pilus-like structures. Pili were described in three distinct variant groups (P1-1, P1-2a and P1-2b) and appear to play a key role in both adhesion and attachment of the bacteria to the host cells, being currently seen as important candidates for vaccine development. Our aim was to investigate the distribution of pilus islets among clones defined by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST).

**Methods:** Representatives of a large collection (n = 491) from colonisation and infection were further characterised. A multiplex PCR reaction was set up to detect the presence of the pil-associated sortase genes to discriminate pili variants and another for surface protein profiling (bca, alp2, alp3, alp4, eps and rib genes). Simpson’s index of diversity (SID) was calculated to evaluate the diversity found among the isolates studied. Wallace coefficient (W) was calculated to quantitatively measure the clustering concordance between different typing methods.

**Results:** The SID for the classification in PFGE clones was 90.04% (CI 95% 86.53–93.54) for the isolates tested and 92.29% (CI 95% 91.24–93.35) for the whole collection, indicating that the set of isolates chosen is representative of the overall diversity. The two most prevalent genotypes were P1-1=P1-2a and P1-2a (53% and 37% of the isolates, respectively). The Wallace coefficient relating the PFGE clusters with the presence of pili (W = 0.8627) was higher than for serotype (W = 0.566), indicating that pili are clonally distributed. Furthermore, MLST sequence type (ST) showed an even higher correspondence with pili (W = 0.9263) and also surface protein profile (W = 0.9539), indicating that ST is a very good predictor of pili and surface proteins and suggesting that it provides a better identification of GBS clones.

**Conclusions:** The high Wallace coefficients relating PFGE and MLST with pili type indicate that pili are a clonal property of GBS.
Modified sequential multiplex PCR for determining multiserotype invasive Streptococcus pneumoniae clinical isolates

P. Irawagi, M.J. Torres, E. Cabrera, J.A. Lepe, J. Garanacho, I. Ohando, J. Aznar* (Seville, ES)

Objectives: The objective of this study was to evaluate a modified scheme of the sequential multiplex PCRs that devised the CDC for determining capsular serotypes of Streptococcus pneumoniae (J Clin Microbiol. 2006 44:124–131). The modified method was that the primer combinations used were adapted to the serotype distribution in Seville, Andalucia (Spain).

Methods: Two hundred fifty-seven invasive pneumococcal isolates obtained during 2004 to 2008 were tested using the modified multiplex PCR system, including 102 previously typed paediatric isolates by serological determination of capsular type, and 155 adult isolates. The primers were grouped into seven multiplex reactions, except for the serotype 10 primer that was not included in none of these reactions. Each reaction includes four primer pairs, and an internal positive control, was tested for the genetic loci lvh and rtxA, which have been related with highly virulent L. pneumophila strains. The presence of the genetic loci lvh and rtxA was examined by PCR. Polymorphism method (AFLP), as suggested by EWGLI. Electrophoretic profiles were compared with the GelCompar software (Applied Maths). The presence of the genetic loci lvh and rtxA was examined by PCR. For the diagnosis of patient infection by Legionella, the urine antigen test was applied (Binax).

Results: Cultures from 36 water samples (24.2%) were positive for L. pneumophila serogroup 1. Colony counts ranged from 500 to 304,000 CFU/ft. All 26 isolates that were typed were indistinguishable, suggesting the domination of one clone over the two-year period of the colonisation. Furthermore, the 26 isolates carried both lvh KAI and rtxA. During the study period, nosocomial legionellosis occurred, and with the implementation of appropriate control measures containment of colonisation was succeeded.

Conclusion: Prolonged colonisation of the water system of our hospital by L. pneumophila was probably due to the domination of a single clone. Although this clone carried both lvh KAI rtxA genetic loci, associated

Molecular typing – part 2

the rest were as follows: serotype 1, 12.5%; 3 and 7F, 10%; 19F, 23B, and 9V, 5%; 9N, 18C, 23F and 23A 2.5% respectively. Non-PCV7 serotypes caused 67.5% of IPD. The first isolate of serotype 19A was detected on February 07 then, six 19A strains were isolated in a four month period (October-2007/January-2008). Pulsed field gel electrophoresis patterns of these six 19A isolates were indistinguishable and different to the first isolate. Illnesses of the related 19A strains included empyema (67.6%), pneumonia with bacteremia (16.7%) and bacteremia (16.7%). Respect antibiotic susceptibility, within the entire S. pneumoniae population (n = 40), a 45% were non-susceptible to P (defined as a penicillin MIC of ≥0.12). Among 19A strains 75% were considered as non susceptible. The proportion of CTX susceptible S. pneumoniae was 80% all isolates/87.5% 19A strains. Resistant rates (%all/%19A strains) among nonβ-lactam agents were as follows: E 25/37.5; T E 25/62.5; C 5/0. No resistance to VA or LV was observed

Conclusion: In our area the recent increase in serotype 19A could be explain by introduction of a specific clone responsible of an outbreak of IPD. The proportion of 19A isolates that are nonsusceptible to commonly used antimicrobial was greater than the proportion for other serotypes.

Molecular typing of Legionella pneumophila from a hospital’s water system

S. Vourli*, E. Velonakis, A. Tarpatsi, M. Pantelaki, L. Zerva, A. Vatsopoulos (Athens, GR)

Objectives: Colonisation of water systems of large buildings, such as hospitals, with L. pneumophila, happens frequently and its containment is necessary in order to prevent nosocomial legionellosis. It has been reported that colonisation of a water system for long time periods is related to the persistence of a small number or one clone. The aim of this work was to study the clonality of L. pneumophila isolates that colonised the water distribution system of “Attikon” University Hospital over a two-year period. Additionally, we searched for the genetic loci lvh and rtxA, which have been related with highly virulent L. pneumophila strains.

Methods: 149 water samples were collected from March 2006 to March 2008 and were cultured according to the guidelines of the European Working Group for Legionella Infections (EWGLI). Identification of suspected colonies and serotyping were performed by appropriate latex agglutination kits (Oxoid). Twenty-six isolates, representative of each site and date of sampling, were typed with the Amplified Fragment Length Polymorphism method (AFLP), as suggested by EWGLI. Electrophoretic profiles were compared with the GelCompar software (Applied Maths). The presence of the genetic loci lvh and rtxA was examined by PCR.

Results: Cultures from 36 water samples (24.2%) were positive for L. pneumophila serogroup 1. Colony counts ranged from 500 to 304,000 CFU/ft. All 26 isolates that were typed were indistinguishable, suggesting the domination of one clone over the two-year period of the colonisation. Furthermore, the 26 isolates carried both lvh KAI and rtxA. During the study period, nosocomial legionellosis occurred, and with the implementation of appropriate control measures containment of colonisation was succeeded.

Conclusion: Prolonged colonisation of the water system of our hospital by L. pneumophila was probably due to the domination of a single clone. Although this clone carried both lvh KAI rtxA genetic loci, associated
by previous studies with high virulence, no patients were infected during the two-year colonisation period.

**P1348** Plasmid profile of multiresistant enterobacteria other than *Escherichia coli* in Cantabria, Spain

B. Ruiz del Castillo*, E. Román, A. Carattoli, L. Martínez-Martínez (Santander, ES; Rome, IT)

**Objectives:** The aim of this study was to characterise plasmid of multiresistant enterobacteria other than *E. coli* isolated in our sanitary area.

**Methods:** One hundred consecutive clinical isolates of enterobacteria resistant to \( \geq 3 \) of: expanded-spectrum \( \beta \)-lactams, quinolones, chloramphenicol, gentamicin/tobramycin, and cotrimoxazole isolated in our centre (January 2004 to March 2006) were included. Susceptibility testing and ESBL detection were determined by microdilution (CLSI guidelines), and clonal relationship by Rep-PCR. Plasmids were extracted (Kado-Liu method), analysed by agarose gel electrophoresis and characterised by replicon typing (J Microbiol Methods 2005; 63:219–28), with confirmation of positive results by sequencing.

**Results:** The following species and Rep/PCR patterns were observed: *C. freundii* (n=6; 6 clones), *Enterobacter aerogenes* (2/2), *E. cloacae* (11/6), *Klebsiella pneumoniae* (21/7), *K. oxytoca* (n=1/1), *Morganella morganii* (27/3) and *Proteus mirabilis* (32/16). Plasmids were studied in at least one isolate of every Rep/PCR patterns, and in at least 3 isolates of the same pattern if MICs of at least 2 compounds differed in at least two dilutions. All *E. cloacae* carried IncHI2 plasmids; three of them also carried IncN plasmids. The 7 clonal groups of *K. pneumoniae* produced ESBL and carried plasmids IncFII (positive for repFIIA3), and plasmids of groups IncN (Kp1, Kp7), IncHI1 (Kp4), IncK (Kp5) and IncI1 (Kp 6). The single *K. oxytoca* produced a plasmid-mediated AmpC and carried an IncP plasmid. All *P. mirabilis* isolates carried IncFI plasmids (carrying the repFIC only replicon). One *E. aerogenes* (producing ESBL) carried IncF plasmids, positive for the repFIB and repFII replicons. The following incompatibility groups were identified in *C. freundii*: IncF (positive for repFIC) and IncP (C1), IncHI2 (C2) and IncHI1, repF and IncFIIA (C3). No amplicons by the replicon typing were detected in any of the clonal groups of *M. morganii*, in three clonal groups of *C. freundii* and in one clonal group of *E. aerogenes*. No amplicons by the replicon typing were detected in *E. coli* (except *M. morganii*). Similar plasmids were isolated in the area contains plasmids of different incompatibility groups. Similar plasmids have been isolated in isolates of the same species but of different clonal groups.

**P1349** Replicon typing of plasmids coding for \( \beta \)-lactamases of the CTX-M-9 group or CMY-2 in multiresistant *E. coli* clinical isolates from Cantabria, Spain

B. Ruiz del Castillo*, B. Guerra, A. Carattoli, L. Martínez-Martínez (Santander, ES; Berlin, DE; Rome, IT)

**Objectives:** In an ongoing study on multiresistant *E. coli* producing (n=100) or not (n=100) extended-spectrum \( \beta \)-lactamases (ESBL) isolated in Santander, Spain, 21 clonally-unrelated (as determined by Rep-PCR) isolates producing \( \beta \)-lactamases of the CTX-M-9 group (CTX-M-14 or CTX-M-9; n=16) or CMY-2 (n=5), respectively, were identified. The aim of this study was to characterise the plasmids of these isolates.

**Methods:** BlaCTX-M-9-group and blacMY-2 genes were detected by PCR. Conjugation experiments were made using azide (Az)-resistant *E. coli* J53 as recipient strain. Transconjugants were selected with Az (100 mg/l) and cefotaxime (2 mg/l; blacTX-M-9-group) or ampicillin/gentamicin/sulfamethoxazole (100 mg/l, 8 mg/l and 1000 mg/l; blacMY-2), respectively. Plasmids from both parental strains and derived transconjugants were extracted by the Kado-Liu method, analysed by agarose gel electrophoresis and characterised by replicon typing (J Microbiol Methods 2005; 63:219–28), with confirmation of positive results by sequencing. The relationship between replicon units and \( \beta \)-lactamase genes in the same plasmid was assessed by Southern blot hybridisation using specific DNA probes.

**Results:** Plasmids of several sizes (around 100–150 kb) were detected in all 21 clinical isolates evaluated. Transconjugants were obtained from all 16 isolates with CTX-M-9 group and from 3 out of the 5 with CMY-2 plasmid analysis showed that in 13 of 16 cases the gene coding for the blactx-m-9-group enzyme was located on conjugative IncK plasmids, and in the remaining 3 cases on IncI1 plasmids. In 2 and 1 out of 3 transconjugants with blacMY-2, this gene was on IncA/C2 and IncI1 plasmids, respectively.

**Conclusions:** In our area, we have observed an association of certain \( \beta \)-lactamase genes with specific large conjugative plasmid: those coding for blactx-m-9-group are of IncK and IncI1 incompatibility groups, while blacMY-2 is related to plasmids of IncA/C2 and IncI1 groups.

**P1350** Characterisation of extended-spectrum \( \beta \)-lactamase-producing *Klebsiella pneumoniae* isolates causing an outbreak in an intensive care unit

T. Halaby*, R. Muts, C. Blijdens, A. van der Zanden, R. Hendrix, W. Goesens (Enschede, NL)

**Objectives:** The prevalence of ESBL-positive *K. pneumoniae* (ESBL-Kp) as a cause of nosocomial outbreaks in intensive care units is high. In this study the genetic relatedness and the characterisation of ESBL genes of ESBL-Kp isolates involved in a large outbreak in an intensive care unit (ICU) are described.

**Methods:** Isolates were obtained by surveillance cultures taken on admission and during hospitalisation twice a week. For the present analysis only one ESBL-Kp isolate per patient was included. VITEK 2 was used for identification and susceptibility testing. Phenotypic ESBL confirmation was performed by the double disk method. ESBL and integron characterisation was performed by PCR. Diversilab® and Multi Locus Sequence Typing (MLST) were used as genotyping methods.

**Results:** Between August 2001 and January 2008, 191 ESBL-Kp positive patients were identified. 130 stored strains were included for analysis. 118 were identified as *K. pneumoniae*. The majority of the 118 strains were resistant to 3rd generation cephalosporins, gentamicin, tobramycin, amikacin and ciprofloxacin. ESBL PCR was performed on 107 of the 118 strains. In 79 isolates the SHV-amplicon was digested with NheI and it was therefore regarded as ESBL-positive. 107 out of 118 showed the presence of an integron with an ampicillin size corresponding with the aadB gene. By Diversilab® all 118 ESBL-Kp strains clustered in the same group. Diversilab® furthermore revealed some other small groups, consisting of ESBL-Kp strains found in the same ICU, but not related to the large cluster. With MLST performed on a subset of 24 strains identical results were obtained compared to the Diversilab® results.

**Conclusions:** The majority of the patients and isolates involved in the outbreak at the ICU appeared to be closely related as demonstrated by Diversilab® and confirmed by MLST. Diversilab® is useful in investigating genetic relatedness of suspected outbreak strains.

**P1351** An outbreak of multidrug-resistant *Acinetobacter baumannii* in an intensive care unit: the usefulness of Diversilab® in studying clonal relatedness

T. Halaby*, W. Goesens, C. Blijdens, R. Hendrix, A. van der Zanden (Enschede, NL)

**Objectives:** Genotyping is an essential epidemiological tool to aid in outbreak investigations and to determine the genotypic relatedness among isolates, which is important for establishing the sources and modes of transmission of the outbreak strains. In this study the usefulness of the Diversilab® was evaluated during an outbreak of MDR-Acinetobacter baumannii in an intensive care unit (ICU), and was compared to Random Amplification Polymorphism Detection (RAPD) and selective restriction fragment amplification by amplified fragment length polymorphisms (AFLP) as a reference method.
Methods: The VITEK 2 system was used for identification and susceptibility testing. AFLP, RAPD and Diversilab® were used as molecular genotyping methods.

Results: The outbreak occurred during the first 4 weeks of 2008 and was ended by temporarily closing the ward. Twenty one A. baumannii isolates were included. Fingerprints obtained with AFLP, RAPD and Diversilab® showed identical relatedness. On the basis of these data, 2 distinct groups were identified. Group 1 consisted of 13 identical strains isolated from 5 patients during the outbreak, 7 positive environmental screening cultures, and one patient isolate, known to harbour MDR A. baumannii (MR-AB). Group 2 consisted of 4 identical isolates, all from one patient admitted to the temporary ICU. The remaining 4 isolates showed different patterns, 3 known non-related strains and one (non-MDR) from a patient admitted to the ICU during the outbreak.

Conclusions: Diversilab® provides strain discrimination identical to that obtained by RAPD and AFLP. All three methods demonstrated the genetic relatedness between the strain from the suspected index patient, the environmental contamination with the same strain and the strains detected in the 5 patients involved in the outbreak. Furthermore, the data indicated that group 2 included a non-related strain rather than spread of the epidemic strain from the ICU to the temporary ICU. Diversilab® makes molecular typing feasible in laboratories that lack equipment and experience regarding different conventional typing methods.

Rapid and effective analysis of nosocomial outbreaks using the Diversilab semi-automated repetitive sequence-based PCR test system

A.J. Grisold*, G. Zarfeli, V. Strenger, G. Feierl, V. Dosch, E. Leitner, L. Masoud, U. Wagner-Ebel, M. Hoenzl, E. Marth (Graz, AT)

Objectives: Rapid and sensitive methods are essential for typing and monitoring of hospital associated infections. The new Diversilab system (BioMerieux) exhibits a semiautomated repetitive-sequence-based polymerase chain reaction (rep-PCR) for typing. Aim of the study was to evaluate the usefulness and accuracy of the Diversilab system for bacterial strain typing and determination of genetic relatedness of strains associated with nosocomial outbreaks and integration of the system in the workflow of a routine microbiological laboratory.

Methods: For evaluation 20 Methicillin-resistant Staphylococcus aureus (MRSA), 26 multi-drug resistant (MDR)-Acinetobacter baumannii strains and 43 extended-spectrum-beta-lactamase producing Klebsiella spp. strains (30 K. pneumoniae and 13 K. oxytoca strains) from well-defined nosocomial outbreaks were typed using rep-PCR on the Diversilab system. As reference method for bacterial strains-typing pulsed-field gel electrophoresis (PFGE) was performed.

Results: Concerning the 20 MRSA isolates, there was identical cluster formation in both, the Diversilab and the PFGE systems. The same result could be observed in the 26 multi-resistant A. baumannii strains, where strains showed identical cluster formation (into 3 separate clusters) and very similar dendrograms. The 30 ESBL-positive K. pneumoniae strains originated from two chronologically separated nosocomial outbreaks. PFGE placed 28 of the ESBL-positive K. pneumoniae strains into the same cluster, whereas PFGE defined all strains as indistinguishable. The Diversilab-system also formed a cluster of these 28 strains, but the strains in this cluster were not defined as identical, but similar. Both systems identified 2 K. pneumoniae strains as non-outbreak strains. Results from the K. oxytoca outbreak provide similar minor differences between PFGE and results obtained with the Diversilab system, whereas rep-PCR on the Diversilab system exhibits slightly more discriminatory power than PFGE.

Conclusion: The Diversilab system is a rapid, semi-automated repetitive sequence-based PCR test system for typing and analysing bacterial strains, including fungi.

- Compared to PFGE, this study confirms the good discriminatory power of the Diversilab system.
- Results on the Diversilab system could be obtained in a short period (8-24h), which is an essential advantage in rapid analysis of hospital associated infections.

Optical typing in bacterial epidemiology using Raman spectroscopy


Objectives: Raman spectroscopy is a non destructive optical technique capable of providing detailed biochemical information on the molecular composition of analyzed samples. Due to recent optimisation and automation, this method has shown to be sufficiently powerful to discriminate between strains within a species. This discriminatory power combined with a very short processing time and high reproducibility makes the technology ideally suited for microbial typing.

Methods: All isolates were cultured for 20hr on Trypticase Soy agar plates. Biomass was suspended in 10 microliter of sterile distilled water, transferred onto a quartz slide and allowed to dry. Spectroscopic fingerprints were obtained using a dedicated Raman spectrometer, requiring 10 to 80 seconds per sample. Cluster analysis on these fingerprints was performed using the pair wise correlations as a distance measure in combination with Ward’s cluster algorithm.

Results: Technological proof-of-principle for efficient, reproducible typing has been obtained for Staphylococcus species, Acinetobacter species, Klebsiella pneumoniae, Escherichia coli and Enterococcus faecium. The discriminatory power for these species matches that of established genotyping techniques such as pulsed field gel electrophoresis (D-values >0.95). The Raman clustering of isolates was reproducible to the strain level for independent cultures. The typing information gathered with this technique is comparable to information obtained by genotyping methods, but available in a fraction of the time.

Conclusion: Using Raman spectroscopy as a typing method, a significant decrease in turn-around time can be achieved. This allows infection control professionals to act in a timely manner and thus prevent the transmission of microorganisms in an early stage by the implementation of adequate hygienic measures. Therefore we conclude that Raman spectroscopy is an easy-to-use and rapid alternative in the battle against hospital acquired infections.

Molecular and epidemiological plasmid profile analysis of Enterococcus faecalis isolates

E. Wardal*, E. Sadowski, W. Hryniewicz (Warsaw, PL)

Objective: The aim of the study was to analyze the correlation between different types of plasmid profiles obtained from various investigation approaches such as PCR-based replicon typing, PCR-based relaxase and stabilisation/partition modules detection and PFGE-S1 visualisation of plasmid molecules and to compare them with selected epidemiological data of investigated isolates.

Methods: The heterogenous group of 152 E. faecalis isolates comprising 52 different STs has been chosen for the investigation. They were representatives of the pool of isolates collected in the National Medicines Institute in Warsaw from several Polish hospitals during 10 year period (between 1996 and 2005). Among them invasive (n>54), non-invasive (n>67) and carrier isolates (n>28) were present. Replication, stabilisation/partition and relaxase genes typical for different groups of plasmids were detected by PCR. The allelic profile of amplified genes were determined by sequence analysis. Megaplasmids were visualised by PFGE-S1 typing method. Pearson correlation coefficient and chi-square tests were used to calculate significance of association of data.

Results: There were 11 different rep genes, 3 different relaxase genes and 1 toxin gene detected by PCR among investigated isolates. Majority of isolates showed the presence of >1rep gene and >1 relaxase gene. 33 rep profile types were proposed based on the combination of rep genes present in each isolate. Sequence analysis showed the greatest diversity of alleles (n>27) within group of rep genes typical for pheromone responsive plasmids. The number of plasmid bands visualised by PFGE-S1 were highly positively correlated with the number of different rep genes per isolate as well as with the number of relaxase genes. The number of rep genes as well as the number of plasmid bands detected
by PFGE-S1 was the highest among carrier isolates. The results for relaxase and stabilisation/partition modules detection showed statistically significant higher number of these genes among invasive and carrier strains.

**Conclusion:** Variable analysis of plasmid profile and content showed important correlation between PCR-based replication genes detection and the number of plasmids detected by PFGE-S1 typing. The highest number of plasmids and rep genes present among carrier isolates may reflect their role as important source of mobile genetic information.

**[P1355] Polymorphisms of the fimbrae fim2 and fim3 genes in the Finnish Bordetella pertussis population**

*T. Kallonen*, J. Mertsola, Q. He (Turku, FI)

**Objectives:** Bordetella pertussis, the causative agent of pertussis produces three distinct serotypes: Fim2, Fim3 or Fim2,3. These fimbrae are coded by the fim2 and fim3 genes, level of fimbral expression determines the serotypes of *B. pertussis*. The fimbrae elicit type-specific immunity. In Finland, *B. pertussis* Fim2 strains were found to be the most common serotype when 1109 clinical isolates from 1974 to 2006 were studied. Emergence of Fim3 strains started in 1999 and coincided with nationwide epidemics. So far two fim2 (fim2−1 and fim2−2) and four fim3 (fim3−1 to fim3−4) alleles have been found. The different alleles are due to single nucleotide polymorphism(s) in fim2 and fim3 genes which result in amino acid substitution(s). However, polymorphisms of the fim2 and fim3 genes have been only reported in a few countries. In this study we analyzed the fimbral gene alleles of Finnish *B. pertussis* isolates collected since 1990.

**Methods:** PCR-based sequencing was performed for the previously reported polymorphic regions of the fim2 and fim3 genes. For fim2 gene 43 isolates from 1991 to 2005 were studied, and for fim3 gene 128 isolates from 1992 to 2006 were studied. All the isolates were randomly selected from the strain collection of Pertussis Reference Laboratory of the National Public Health Institute, Turku, Finland. The international reference strain Tohama I and the two Finnish vaccine strains were also tested.

**Results:** For fim2 gene, all Finnish isolates tested as well as the reference strain Tohama I had fim2−1. For fim3 gene, two alleles fim3−1 (23%) and fim3−2 (77%) were detected. Before 1999, all isolates had fim3−1. The allele fim3−2 emerged in 1999 and became predominant since then. The two Finnish vaccine strains with serotypes Fim2,3 and Fim3 represented fim2−1/fim3−1 and fim3−1.

**Conclusion:** Polymorphism was found in fim3 gene, but not in fim2 gene, of Finnish *B. pertussis* isolates. Emergence of fim3−2 alleles started in 1999 and coincided with nationwide epidemics. The possible impact of different fim alleles on protective immunity needs further investigation.

**[P1356] Usefulness of automated ribotyping to type Corynebacterium striatum isolates**

*E. Carretto*, D. Barbarini, A. Grosini, C. Farina on behalf of the APSI Study Group

**Objective:** in the past 5 years, *Corynebacterium striatum* (CS) was increasingly reported in different Italian hospitals as the cause of severe diseases (pneumonia, wound infections) in immunocompromised patients or admitted to ICUs. Recently, we performed molecular characterisation of different multidrug-resistant strains of CS. Pulsed-field gel electrophoresis and automated ribotyping allowed us to confirm the presence of a single clone, possessing erm(X), tetA/B, cmx/A/B, and aphA1 genes, but few related subclones (Campanile et al., EID 2009, 15: 75−8). To further investigate the usefulness of the automated ribotyping for typing CS strains, we analysed isolates collected in different Italian hospitals belonging to the APSI (Associazione per la Prevenzione e lo Studio delle Infezioni, Italy) study group.

**Methods:** the Riboprinter Microbial Characterisation System® (Qualicon, Wilmington, USA) was used to perform the strains’ ribotyping. Two reference strains (DSM 20668T and DSM 7185) and a set of 16 clinical isolates collected in different periods were analysed using EcoRI, BstEII and PvuII as restriction enzymes. Among the clinical isolates, some strains that we previously documented belonging to the same clone were included as internal control. The reproducibility of the patterns obtained was evaluated with repeat testing of 8 of isolates randomly chosen. All strains were stored at −80°C until use.

**Results:** among the restriction enzymes used, EcoRI allowed the best discrimination. This enzyme generated more complex fingerprints with 5−7 bands, whereas BstEII and PvuII generated 3−5 band profiles. As expected, using EcoRI the fragment comprised in the range 6−15 kbp allowed a good discrimination among the tested strains, whereas the bands with lower molecular weight appeared to be more conserved. EcoRI distinguished 7 different ribotypes with a good agreement with the internal controls, whereas both BstEII and PvuII differentiated only 6 ribotypes.

**Conclusions:** the increasing reports of infections caused by multidrug-resistant CS in our country is responsible for the awareness that led to the subsequent in-depth examination of these strains. The APSI study group allowed us to collect, to date, more than 150 CS isolates from different Italian hospitals. This preliminary study points out that automated ribotyping using EcoRI could be a good first line typing method to analyze the circulation of CS strains in different geographical areas of our country.

**[P1357] cDNA-AFLP strategy applied in the search of drug resistance markers in medically important fungi**

*V. Lesteroa*, N. Brankova, K. Tankova, S. Panaistou (Sofia, BG)

AFLP (Amplified Fragment Length Polymorphism) is a whole genome analysis technique applied for typing of strains. Both modifications of the technique, DNA and cDNA-AFLP are highly reproducible typing methods.

**Objective:** Development of cDNA-AFLP strategy applied in the search of antimycotic drug resistance markers(s) in medically important fungi.

**Methods:** Clinical *Candida* isolate, susceptible to azole antymycotics was subcultured on agar plates with increased concentrations of tested antymycotics (fluconazole, and ketoconazole). Six drug resistant mutants were subject to DNA- and cDNA-AFLP typing. The six mutants originated from plates with gradually increased concentration of fluconazole and ketoconazole. BamHI, PstI, MboI and HindIII restriction enzymes, appropriate adaptors for the restriction sites, adapter primers and amplification conditions have been tested with the aim to identify the characteristic AFLP patterns. Mutant genotypes were compared with the original drug sensitive strain.

**Results:** DNA-AFLP typing strategy was used with the aim to evaluate possible microevolution in the original and mutant strains. AFLP patterns demonstrate low level of microevolution of the strains after subculturing on antymycotic containing plates. Only few bands showed polymorphisms under this selective pressure. Differential expression of several RNA products were observed by cDNA-AFLP typing.

**Conclusions:** cDNA-AFLP, is an AFLP-based transcript profiling method. It was applied for genome-wide expression analysis in medically important *Candida* species. The technique offers the possibility to analyze organisms without the need for prior sequence knowledge. In essence, the cDNA-AFLP method involves reverse transcription of mRNA into double-stranded cDNA, followed by restriction digestion, ligation of specific adapters and separation of this mixture of cDNA fragments on an automated fluorescently based system. The differential RNA expression profile is linked to drug resistance as showed by the differential expression of few RNAs. Observed differences in band intensities between samples provide a good measure of the relative gene expression level. Identification of differentially expressed genes can be accomplished by purifying cDNA-AFLP fragments from sequence gels and subsequent sequencing. This method has found to be an attractive technique for gene discovery of genes involved in antymycotic resistance in medically important fungi.
Molecular epidemiology of invasive meningococcal disease in the Czech Republic

J. Kalmašová, M. Musilek, P. Kríž (Prague, CZ)

Objectives: Enhanced surveillance of invasive meningococcal disease (IMD) has been conducted in the Czech Republic since 1993, when a new hyperinvasive clonal complex, cc11, emerged and caused increase in IMD incidence and morbidity. Molecular methods for the characterization of Neisseria meningitidis which have been used continuously in the National Reference Laboratory for Meningococcal Infections (NRL) allow precise assessment of the epidemiological situation. The aims of this study were to identify possible epidemiological links between IMD cases across districts of the country, to detect secondary IMD cases, and to assess possible epidemiological links between patients and healthy contacts.

Methods: Epidemiological and microbiological data from the surveillance database for 2007 were analysed. All meningococcal isolates from IMD cases (43 isolates) and healthy contacts (38 isolates) referred to the NRL were characterised by serogrouping, PorA and FimA sequencing (http://neisseria.org/nm/typing/) and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/).

Results: IMD in the Czech Republic in 2007 was caused mainly by serogroup B (67.4%), followed by serogroups C (20.9%) and Y (9.3%). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively). Most isolates from healthy contacts were either serogroup B (63.1%) or non-groupable (31.6%). They showed high heterogeneity in clonal complexes, with the prevalence of cc41/44 (18.6%, 13.9%, 9.3% and 9.3%, respectively).

Conclusion: No secondary case of IMD has been detected in the Czech Republic. The measures taken in IMD foci are efficacious and need to be targeted at close contacts only.

Genotypes of Coxielia burnetii. Comparison between AFLP and VNTR typing strategies

S. Punatot, N. Brankova, V. Leuterova, P. Butaye, W. Vanderhaegen, R. Toman (Sofia, BG; Brussels, BE; Bratislava, SK)

The accurate typing is a difficult part in the epidemiological research. Selection of the typing strategy for bacteria has been one of the most important practical questions to be solved within the bacteriological laboratories.

Objective: To compare typeability of VNTR and AFLP techniques applied for Coxiella burnetii.

Methods: We investigated the possibility to apply the AFLP technique for typing of the C. burnetii isolates collected in Europe and North America. BamHI, PstI, MboI and HindIII restriction enzymes, the appropriate adaptors for the restriction sites, and both adaptor primers and amplification conditions have been tested with the aim to identify the characteristic AFLP patterns. The BamHI/PstI combination showed promising results. The PCR amplified Cy-5 fluorescently labelled restriction fragments were separated in an automated sequencer. The obtained restriction patterns were elaborated with the GelComparII software. Seven polymorphic VNTR markers were selected for typing. The typing results demonstrated homogeneity in the genotypes from Slovakia and a good distinction from the North American C. burnetii genotypes. Only two VNTR markers demonstrated polymorphisms in the Slovak isolates.

Results: The VNTR typing demonstrated that the C. burnetii isolates from Slovakia form a quite homogeneous genotype cluster. Both typing methods, VNTR and AFLP, have demonstrated that the C. burnetii genome is conservative and evolutionary stable. The generation of polymorphisms is not dynamic. It appears that C. burnetii is not rich in the BamHI and PstI recognition sequences. The AFLP pattern demonstrates a relatively small number of amplified fragments in comparison to Chlamydia trachomatis. HindIII/MboI gives significantly more fragments. A small number of them are polymorphic.

Conclusions: VNTR identifies five clusters. All Slovak isolates belong to cluster 5. The proposed AFLP methodology identifies only three clusters. In the same time, the AFLP has identified unique “Slovak” AFLP pattern not found in other isolates. Results of DNA digesting with HindIII and MboI restriction enzymes repeat both results and conclusions found for BamHI and PstI. Both AFLPs confirm that two isolates, one from Slovakia and one from the USA are different from others. Based on the AFLP results of the investigated C. burnetii isolates a specific PCR could be developed for unique marker(s).

Direct genotyping of Coxiella burnetii in human and animal clinical samples


Objectives: Studies towards the molecular epidemiology of Coxiella burnetii are hampered by the availability of sufficient material for analysis. So far, molecular fingerprinting of C. burnetii has relied on cultivation/enrichment of the pathogen prior to analysis which requires specific biosafety facilities. Molecular diagnosis of Q-fever has mainly focussed on the use of serum/plasma for analysis. We explored the direct genotyping of C. burnetii on various human and animal clinical samples from the current and ongoing Q-fever outbreak in The Netherlands.

Methods: A 6-marker MLVA panel (selected from Arricau-Bouvery et al., 2006) distributed over 2 multiplex PCR reactions was applied to DNA extracted from serum/plasma, sputa, faeces, urine, throat-swabs and genital swabs. PCR products were analysed by multicolour capillary electrophoresis. Repeat numbers were deduced from the fragment sizes relative to those obtained using the well characterised and sequenced Nine-Mile strain. The relative DNA load of C. burnetii in these samples was determined using real-time PCR targeting the IS1111a element.

Results: Multiple different genotypes were obtained in those clinical samples with the highest DNA loads (Ct value < 35). These clinical samples included all of the above mentioned sample materials. Partial genotyping results were obtained in some of the samples with a lower DNA load. Certain genotypes were found in both human and animal samples. Despite several minor differences, all genotypes were very similar indicating a possible clonal origin of the outbreak.

Conclusions: Direct genotyping may be a promising and welcome addition to the current techniques used to study the molecular epidemiology of C. burnetii and to identify the possible origin of human Q-fever infections.

Molecular typing of Listeria monocytogenes virulent serotypes

M.A. Ferrus*, Y. Moreno, L. Ballesoteros, A. Rodrigo, G. Cuesta, J. Hernández (Valencia, ES)

Objectives: Listeria monocytogenes is the causal agent of one of the most important foodborne diseases worldwide. Pregnant women, newborns and immunocompromised persons are especially susceptible to the infection, with a case-fatality rate until 75%. L. monocytogenes presents a great strain virulence variation. Among the 14 known serotypes, only three (1/2a, 1/2b and 4b) produce 95% of the infection cases. So, molecular methods which can differentiate among strains...
Molecular typing of human isolates of *L. monocytogenes*

**Material and Methods:** Ninety-five isolates were used for typing. The genomic DNAs were obtained by enzymatic digestion with three enzymes: ApaI, AscI, and SmaI. The restriction profiles were determined by API-Listeria system. Serotyping was performed by heat-inactivated bacteria agglutination with the commercial system Listeria antisera set (Denken Seiken, Co., Ltd., Tokyo).

**Results:** All the isolates were analysed with an automated repetitive element-based PCR (rep-PCR) system. Results of the similarity analysis revealed four primary genetic groups among the isolates, separated at a relative similarity of 85%. *Listeria* species other than *L. monocytogenes* had similarity scores of less than 70%, and could be easily distinguished from *L. monocytogenes* isolates. No relationship could be found between the serotype and the rep-PCR pattern. However, distinct biotypes were found in the different genetic groups, with a statistically significant distribution.

**Conclusion:** This study shows that the automated REP-PCR system possesses a great discriminatory ability for subtyping *L. monocytogenes*. This rapid method may be useful for species identification and could be considered as an alternative method for epidemiological tracking of *L. monocytogenes* virulent serotypes.

**Acknowledgments:** This work was supported by AGL2005-07776-C03-01 and AGL2008-05275-C03-02/ALI grants from Ministerio de Ciencia y Tecnología, Spain (National and FEDER funds).

**Objectives:** *Listeria monocytogenes* is a Gram-positive intracellular bacterial pathogen responsible for serious infections in immunocompromised individuals, pregnant women, and neonates. Current surveillance schemes assume that all the isolates of *L. monocytogenes* are equally pathogenic, but several observations have suggested that virulence varies from one strain to another. Therefore, the ability to differentiate strains of *L. monocytogenes* is particularly important for tracking transmission of pathogenic strains within food-processing plants and developing more effective intervention strategies to prevent recalls and human illness. The present study was carried with the aim of analysing biochemically, serologically and molecularly 15 human isolates strains of *L. monocytogenes*.

**Methods:** A total of 15 *L. monocytogenes* strains were isolated from human sources at the Microbiology and Virology Department, Policlinico di Bari, Italy. They were serotyped by commercially available listerial O and H antisera and characterised by PFGE method with three different restriction enzymes, Apal, Ascl, and Smal.

**Results:** All the strains were confirmed by conventional biochemical tests as *L. monocytogenes*. The prevalent serovars were 4b (60%) and 1/2a (40%). The genomic DNA restriction profiles of the 15 *L. monocytogenes* isolates obtained after digestion with endonucleases resulted similar. Only one strain presented a different electrophoretic pattern using all three enzymes. This strain prA gene, transcriptional activator of the majority of virulence genes, was amplified and sequenced. This gene presented multiple silent mutations and one not conservative mutation that transforms Hys181 in Leu. This mutation could probably explain the different degree of pathogenicity of this strain that caused a severe disease in a not immunocompromised patient.

**Conclusion:** The results so obtained confirm that these molecular subtyping methods can usefully support the epidemiologic and laboratory investigation of *L. monocytogenes* infections. They allow to establish specific relationships between different isolated strains and their pathogenic grade.

**Objectives:** *Chlamydia trachomatis* is one of the most prevalent sexually transmitted bacterial pathogen. Serovars D to K are commonly associated with urogenital infections in males such as urethritis and epididymitis. Lymphogranuloma venereum (LGV), a sexually transmitted disease (STD) caused by *C. trachomatis* serovars L1, L2 and L3, is endemic in tropical countries but from the year 2004 there are reported cases in Europe. We try to study the circulating genotypes in a group of male patients coming from a STD clinic in our city.

**Methods:** We undertook a two years review, from January 2007 to December 2008. We included 84 male patients coming from a STD clinic (median age was 29.7 years). To detect bacterial DNA in clinical specimens, the COBAS TaqMan CT Test (Roche) was used. To genotype bacterial strains, a 990 bp-fragment of ompA gene was amplified by a nested PCR. The amplicons were purified by using Montage DNA Gel Extraction Kit (Millipore) and sequenced with BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The individual sequences were compared to those available in the GenBank databases with the BLASTN program run on the NCBI Server.

**Results:** Twenty-two out of 84 (26.2%) rectal swabs positive for *C. trachomatis* were found. Sixteen of 22 (72.7%) *C. trachomatis* positive samples were genotyped. The most prevalent genotype was E (50%) followed by D (18.5%), G (12.5%) and J (12.5%). The median age was 31.62 years. HIV status was known for 2 patients (12.5%) and another concomitant sexually transmitted disease was present in 6 cases (37.5%): 1 syphilis (6.25%), 3 human papillomavirus (HPV) type 6 (18.75%) and 2 HPV type 11 (12.5%).

**Conclusion:** Genotype E is the most frequent in this group of patients. Genotypes distribution is similar to other reports. Any *C. trachomatis* variant related with LGV was not found.

**Introduction:** *Chlamydia trachomatis* is the most common cause of bacterial sexually transmitted infections (STI) worldwide. Besides, *Chlamydia* acts as a marker of increased risk for HIV infection. There are 19 serovars of *C. trachomatis*, as defined by serological responses to the outer membrane protein (ompA) and can be conveniently defined by sequence analysis and genotyping of the ompA gene. There has been significant interest in whether these genotypes are differentially associated with clinical manifestations, geographic locations or gender.

**Objective:** The aim of this study was to explore the distribution of these genotypes in a group of patients referred from Bilbao health area located in the North of Spain.

**Material and Methods:** One hundred clinical samples from different anatomical sites sent to the Basurto Hospital STD Laboratory for assessment between January 2007 and June 2007 were screened as positive for *C. trachomatis* by polymerase chain reaction (PCR) using in-house real-time PCR assay targeting the cryptic plasmid. Direct real-time PCR assay was used to produce two 480-bp amplicons defining the V1-V2 and V3-V4 regions of the ompA gene using the primers previously described by Dean et al. The PCR reaction was carried out in a volume of 20 µl and was performed in a LightCycler 480. The final product was purified using UltraClean PCR purification system and sequenced using BigDye Terminator Version 3.1 chemistry.
according to the kit instructions. Sequencing reactions were purified with AutoSeq G-50 (GE Healthcare) and sequenced on an ABI 3130 Genetic Analyzer. DNA sequences obtained were aligned to obtain full-length sequence information of each sample and queried against the BLAST database.

**Results:** 82 (82%) of the specimens tested contained only one serovar and 18 (18%) contained two or three serovars. In common with studies from other countries, genotypes E and F were the dominant strains found in our patient population. The other genotypes distribution was the following: K (13.41%), D (12.19%), G (10.97%), Ja (9.75%), I (7.31%), H (10.97%), Ia (4.87%), L2 (1.21%), and L2b (3.66%). Patients with *Chlamydia trachomatis* type G were significantly older than the mean of the other groups. This result could suggest that the immune response to E drives a population switch to the G genotype with repeated exposure as pointed out in other studies, but further research in this point is necessary.

The most prevalent genotype in Osijek-Baranja County was the following: K (13.41%), D (12.19%), G (10.97%), Ja (9.75%), I (7.31%), H (10.97%), Ia (4.87%), L2 (1.21%), and L2b (3.66%). Patients with *Chlamydia trachomatis* type G were significantly older than the mean of the other groups. This result could suggest that the immune response to E drives a population switch to the G genotype with repeated exposure as pointed out in other studies, but further research in this point is necessary.

**Methods:** Genetic material was isolated by using Genomic Mini AX BACTERIA kit (A&A Biotechnology, Poland). In order to obtain amplification products, RAPD-PCR method with primers: 272, 208, ERIC 2 and PAL 2 was used. With the help of agarose gel electrophoresis with EtBr, reaction products were exposed. The result was analysed with computer program BIO-PROFIL BioID++ (Vilber Lourmat, France). Genetic similarity between strains was viewed by using dendrograms. The dendrograms with UPGMA clustering method were received.

**Result:** As a result of bacteria genotyping, the genetic similarity value of specified clonal groups, was equal for the following primers: *P. aeruginosa:* 272−47%, 208−71%, ERIC 2−46%, PAL 2−49%, *A. baumannii:* 272−63%, 208−59%, ERIC 2−52%. The homology value between strains was also evaluated. Isolates were closely related if the genetic similarity degree was in the range of 100−85%. In the group of *P. aeruginosa* isolates, for primers 272 and 208, one clonal group with the high level of homology between strains was specified (approximately isolates 5, 6 and 2, 3). For the primer ERIC 2 one clonal group (5, 6) and one pair of strains with the same genetic profile (7, 8) were observed. The primer PAL 2 viewed high similarity degree for 3 pairs of isolates (1, 2; 4, 7; 6, 8).

In the group of *A. baumannii* strains, for the primer 272, three clonal groups were specified: 1, 2; 8, 10, 11; 12, 14. The primer 208 let specify four clonal groups: 5, 10; 8, 11; 2, 3, 6, 9; 12, 13, however ERIC 2−two groups with high homology value between strains.

**Conclusion:** *P. aeruginosa:* all analysed isolates probably derived from several subpopulations of a hospital strain, which has undergone evolutionary divergence phenomenon in time, as a result of medicine application and changing conditions of the hospital environment. *A. baumannii:* there was observed higher degree of homology between isolates. All strains have hospital nature, except isolate number 7. A lack of high similarity degree with the rest of strains, can indicate posthospital origin.

**P1356 Detection and genotyping of Chlamydia trachomatis in clinical urogenital samples from north-eastern Croatia**

Z. Bosnjak*, D. Vukocić, N. Ruzman, I. Roksandić-Krizan, M. Peric, S. Đezjan (Osijek, HR)

**Objectives:** Due to the chronic and “silent” infection and variable antigenic structure of the *Chlamydia trachomatis* we supplemented Direct Immune Fluorescence with the molecular diagnostic method using COBAS TaqMan 48 real time PCR instrument. The fast molecular diagnostics of *C. trachomatis* infections and adequate therapy of the infected individuals are the crucial step in the C. trachomatis spread control. The epidemiological data obtained from the Osijek-Baranja County Institute of Public Health archives revealed the true number of chlamydial genital infections among other genital infections in the last five years in our county.

The aim of the present study has been to reveal the most prevalent serotype of the *C. trachomatis* detected in urine and gynaecological samples in the population of the Osijek – Baranja County and to monitor the infection and the therapy efficiency. The determined serotype distribution has been compared with the *C. trachomatis* distribution pattern in other regions of the World. All the samples were collected from the Osijek-Baranja County Institute of Public Health and gynaecologist’s offices.

**Methods:** COBAS TaqMan CT test is an in vitro nucleic acid amplification test which utilises real time PCR technology. The test has been developed to confirm analogous detection of all 15 *C. trachomatis* serotypes and it produces results within 3 hours. Subsequently all the positive samples have been analysed directly by sequencing of the amplified omp1 fragments using Applied Biosystems 3130 Genetic Analyser. Genotyping and sequence mutation analysis have been performed by BLAST searching and compared with the reference sequences of all known *C. trachomatis* serotypes.

**Results:** The most prevalent genotype in Osijek-Baranja County was serotype E (in agreement with Sweden and Taiwan data), followed by F, G, D, K, J, H, B, and Ia (differs from Sweden and Taiwan data). The sequence of omp1 gene showed limited variation.

**Conclusion:** The obtained omp1 gene genotyping results could be useful tool for epidemiological characterisation of circulating *C. trachomatis* in our community. Further analyses in this ongoing project might also reveal the influence of certain serovar on clinical signs, intensity of infection, duration of concomitant infection considering age and sex and therapy efficiency, as well as possible presence of chlamydial coinfection and reinfection.

**P1365 Molecular analysis of new variant of Encephalitozoon cuniculi responsible for disseminated microsporidiosis in a renal transplant recipient**

H. Talabani, C. Sarfati, E. Pilebou, T. van Gool, F. Derouin, J. Menotti* (Paris, FR; Amsterdam, NL)

**Objectives:** To identify and subtype a microsporidian strain responsible for disseminated infection in a renal transplant recipient presenting fever and abdominal pain.

**Patient and Methods:** A 38-year-old female renal transplant recipient presented fever, cough, non specific abdominal pain and anorexia 4 months after transplantation. No bacterial aetiology was found. After a 1-month hospitalisation, many spores of microsporidia were detected in sputum, urine and kidney biopsy. For species diagnosis, specific PCR amplifying *Enterocytozoon bieneusi* internal transcribed spacer (ITS) and *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi* and *Encephalitozoon hellem* small subunit rRNA genes were performed. Indirect immunofluorescence technique (IFAT) was used to search for antibody responses against the spore wall and the polar tube of *E. cuniculi*. Subspecific molecular typing was made by PCR and sequence analysis of a 403-bp DNA fragment containing *E. cuniculi* ITS.

**Results:** Specific PCR for *E. bieneusi*, *E. intestinalis* and *E. hellem* were negative, but the presence of *E. cuniculi* DNA was evidenced in urine, sputum and kidney biopsy specimens. An IgG antibody response against the spore wall of *E. cuniculi* were evidenced by IFAT in a serum specimen sampled early after infection. Additionally, an IgG antibody response against the polar tube of *E. cuniculi* was evidenced in a serum specimen sampled 3 months after. Sequence analysis of the DNA fragment containing *E. cuniculi* ITS showed the presence of 5 repeats of 5′-GTTT-3′ in all tested specimens collected from our patient (1 sputum, 1 kidney biopsy and 4 urine samples), which does not correspond to the number of repeats found in the 3 described strains of *E. cuniculi* in the literature.

**P1366 Molecular analysis of Pseudomonas aeruginosa and Acinetobacter baumannii strains, isolated from intensive care unit patients of with hospital-acquired pneumonia**

A. Budak*, D. Wlodarczyk, P. Nowak, P. Mrowiec, M. Wegrzyn (Cracow, PL)

**Objective:** The aim of study was genotypical analysis of *Pseudomonas aeruginosa* (10) and *Acinetobacter baumannii* (14) strains, isolated from tracheal aspirates, which were aetiologic agents of hospital acquired pneumonia in patients of ICU.

**Methods:** Genetic material was isolated by using Genomic Mini AX BACTERIA kit (A&A Biotechnology, Poland). In order to obtain amplification products, RAPD-PCR method with primers: 272, 208, ERIC 2 and PAL 2 was used. With the help of agarose gel electrophoresis with EtBr, reaction products were exposed. The result was analysed with computer program BIO-PROFIL BioID++ (Vilber Lourmat, France).

**Result:** As a result of bacteria genotyping, the genetic similarity value of specified clonal groups, was equal for the following primers: *P. aeruginosa:* 272−47%, 208−71%, ERIC 2−46%, PAL 2−49%, *A. baumannii:* 272−63%, 208−59%, ERIC 2−52%. The homology value between strains was also evaluated. Isolates were closely related if the genetic similarity degree was in the range of 100−85%. In the group of *P. aeruginosa* isolates, for primers 272 and 208, one clonal group with the high level of homology between strains was specified (approximately isolates 5, 6 and 2, 3). For the primer ERIC 2 one clonal group (5, 6) and one pair of strains with the same genetic profile (7, 8) were observed. The primer PAL 2 viewed high similarity degree for 3 pairs of isolates (1, 2; 4, 7; 6, 8).

In the group of *A. baumannii* strains, for the primer 272, three clonal groups were specified: 1, 2; 8, 10, 11; 12, 14. The primer 208 let specify four clonal groups: 5, 10; 8, 11; 2, 3, 6, 9; 12, 13, however ERIC 2−two groups with high homology value between strains.

**Conclusion:** *P. aeruginosa:* all analysed isolates probably derived from several subpopulations of a hospital strain, which has undergone evolutionary divergence phenomenon in time, as a result of medicine application and changing conditions of the hospital environment. *A. baumannii:* there was observed higher degree of homology between isolates. All strains have hospital nature, except isolate number 7. A lack of high similarity degree with the rest of strains, can indicate posthospital origin.
Conclusion: Molecular typing showed that our patient was infected with a previously undescribed variant of *E. cuniculi*, which we propose to name type IV strain.

Epidemiology of MRSA in animals

**P1368 Exposure related carriage of methicillin-resistant Staphylococcus aureus in veal calf farmers in the Netherlands**

H. Graveland*, J. Wajengaard, D. Heederik (Utrecht, NL)

**Objectives:** Recent studies showed that pig and cattle farming is a risk factor for MRSA colonisation in humans. In depth analysis of the specific risk factors associated with MRSA colonisation in veal farmers is lacking. To study the prevalence of MRSA in veal farmers and associated risk factors, we conducted a cross sectional study among 390 farmers and family members. To gain more insight in the dynamics and persistence of MRSA colonisation in this population a longitudinal study is ongoing in which repeated nasal- and throat swabs are being taken, comprising MRSA colonisation in periods with and absence of or reduced animal contact.

**Methods:** 102 veal calf farms were randomly selected and visited from March 2007 – February 2008. Participating farmers were asked to fill in a questionnaire (n = 390) with questions about lifestyle factors (smoking), activities on the farm and contact with animals. A nasal swab was taken from each participant. Swabs were analysed for MRSA by selective enrichment, culturing and confirmed by MecA PCR. Spa types of the isolates were identified. Data were analyzed using multi-level logistic regression analysis to adjust for potential clustering within farms.

**Results:** MRSA prevalence was 33% in veal farmers and 8% in family members. Duration of animal contact was strongly and positively associated with nasal human MRSA colonisation. Additionally, activities with direct animal contact, such as feeding the calves and tasks involving different forms of veterinary care were also positively associated with MRSA colonisation. Furthermore the percentage of MRSA positive calves on the farm is a risk factor. Initial results from the longitudinal study indicates that the prevalence of MRSA colonisation is lower in periods with reduced animal contact.

**Conclusions:** The association between duration of contact with animals and MRSA colonisation indicates that especially intensive contact with animals might put them at risk for MRSA colonisation. However, the large differences in MRSA prevalence between farmers and family members and the strong association with the percentage of positive calves is suggestive of either transient colonisation of NT-MRSA in humans or contamination of the sampled nasal cavities with MRSA containing dust from the stable air instead of carrier ship. Full analysis of the ongoing longitudinal study will gain more insight in the dynamics and duration of MRSA colonisation.

**P1369 Spread of methicillin-resistant Staphylococcus aureus sequence type 398 in Europe**


**Objective:** To estimate the contribution of livestock associated methicillin-resistant *Staphylococcus aureus* Sequence Type 398 (MRSA-ST398) to the burden of MRSA from humans in Europe in 2007.

**Methods:** A cross sectional survey was performed. In September 2008, a questionnaire was sent to 43 laboratories in 23 countries, with questions on general laboratory information, number of MRSA isolates and number of MRSA-ST398 isolates in 2007.

**Preliminary results:** As of December 2008, we have received the replies from 24 (56%) laboratories from 17 (74%) countries. Only results from the 20 laboratories that typed MRSA isolates are reported. This includes data from the following 15 countries: Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Greece, Hungary, Iceland, Italy, Ireland, the Netherlands, Sweden, Switzerland and Turkey.

In total 7,770 MRSA isolates with typing results were reported and 113 (1.5%) were MRSA-ST398. Eight of the 15 countries reported MRSA-ST398. The proportion of MRSA-ST398 ranged from 0 to 11.9%. The countries with the highest proportion of MRSA-ST398 were the Netherlands (11.9%), Belgium (4.7%), Austria (2.7%) and Denmark (1.6%).

**Conclusions:** MRSA-ST398 has spread across Europe, but the proportion of such isolates generally remains low. The highest proportions were reported by the Netherlands, Belgium, Austria and Denmark. Three of these countries, i.e. the Netherlands, Belgium and Denmark, have the highest pig density per square kilometre among European countries.

**Methicillin-resistant Staphylococcus aureus Clonal Complex 398 does not spread from farms into the community**

B. van Cleef*, E. Verkade, M. Wulf, A. Buiting, A. Voss, X. Huijsdens, M. Mulders, J. Kluytmans (Breda, Eindhoven, Tilburg, Nijmegen, Amsterdam, NL)

**Objective:** To determine whether methicillin-resistant *Staphylococcus aureus* Clonal Complex 398 (MRSA-CC398) has spread from livestock farms into the general community in the Netherlands.

**Methods:** A cross-sectional prevalence study was conducted in 3 urban municipalities with the highest densities of pigs in the Netherlands (a). Adult persons (n = 2703) were randomly selected from the national registry of inhabitants. A questionnaire was mailed asking for participation and contact with livestock, working in healthcare, past history of MRSA, contact with MRSA-positive persons and hospitalisation abroad. Furthermore, a nasal swab was taken to determine the presence of MRSA. To determine if spread from farms into the community had occurred, a stratified analysis was done for persons with and without contact with livestock. The sample size was calculated for 450 persons without livestock contact to exclude a prevalence of at least 2%, assuming a background prevalence of 0.5% (c=0.05 and β=0.10).

**Results:** As of December 2008, complete data were collected from 517 individuals (response 19%). All of the 478 persons without contact with livestock tested negative for MRSA. Of the 39 persons who indicated regular contact with livestock (either work at or live on a livestock farm), 8 persons (20.5%, 95% confidence interval 10.8–35.5) tested MRSA-positive. Seven of these 8 persons reported contact with pigs and 1 with poultry. 3/8 had been tested MRSA-positive previously, and 5/8 reported recent contact with MRSA-positive persons. Spa-typing is currently being performed.

**Conclusions:** MRSA-CC398 was not found in persons without contact with livestock. Therefore there are presently no indications that this clone is spreading into the community in the Netherlands. (a) according to the annual count of farming 2005–2007 done by CBS Statistics Netherlands (www.cbs.nl).

**Detection of Staphylococcus aureus multi-locus sequence type 398 (ST398) in blood cultures**

E. Verkade*, A. Budding, V. Weterings, J. van Baal-Vissers, A. Bergmans, P. Szavelkoul, A. Buiting, J. Kluytmans (Breda, Amsterdam, Tilburg, NL)

**Objectives:** Recently a new clone of methicillin-resistant *Staphylococcus aureus* (ST398 MRSA) has emerged which is related to animal husbandry. The methicillin-susceptible ancestor of this strain is a common *S. aureus* variant in pigs. A recent study showed that 2.1% of MSSA strains that caused bacteremia in humans were ST398. This finding is worrying but the study did not use a well-defined collection of strains and thus the results may be biased. Therefore, we determined the prevalence of ST398 MSSA in consecutive bacteremic patients in an area in The Netherlands with a high density of pigs.
Epidemiology of MRSA in animals

Methods: In two time periods all consecutive episodes of MSSA bacteraemia were included. 251 MSSA strains were isolated between 1996 and 1998 in Tilburg and Breda, The Netherlands. Another 210 MSSA strains were isolated between 2002 and 2005 from patients in Breda, The Netherlands. The cities of Tilburg and Breda are located in the Southern part of The Netherlands, which is an area with a high density of pigs.

In order to identify ST398 MSSA, the 16S-23S interspace (IS) region lengths from all isolates were determined using specific primers in a PCR. Every S. aureus strain has 5 or 6 IS regions in its chromosome. The length of the individual regions vary within the chromosome, so when amplified and sorted by length using gel electrophoresis, each strain produces an unique pattern of bands.

All isolates were subcultured to obtain fresh growth. A 1 McFarland suspension was made in 0.75% NaCl suspension. This suspension was centrifuged at 14,000 rpm for 3 minutes in an eppendorf vial. The supernatant was removed and the pellet was resuspended in Aquadest water by vortexing. This suspension was then centrifuged at 14,000 rpm for 3 minutes. The supernatant was used for PCR without further processing. For amplification of the 16S-23S rRNA spacer regions, two primers were constructed in conserved regions of 16S and 23S rDNA respectively. PCR products were separated on a 2% agarose gel. All banding patterns were then visualised using an UV Transilluminator and the unique ST398 banding pattern were checked for the unique ST398 banding pattern. banding pattern were then visualised using an UV Transilluminator and the unique ST398 banding pattern were checked for the unique ST398 banding pattern. It was determined that each ST398 MSSA strain produces an unique pattern of bands. When amplified and sorted by length using gel electrophoresis, each strain produces an unique pattern of bands.

Results: None of the 461 MSSA strains showed a banding pattern that corresponded to that of ST398 (95% confidence interval of 0.00–0.01).

Conclusion: In an area with a relative high density of pigs, ST398 MSSA was not found as a cause of bacteraemia in humans. This finding indicates that ST398 MSSA is not a frequent cause of invasive disease in humans.

Heterogeneity among porcine MRSA ST398 isolates


Objectives: Within the last few years, methicillin-resistant Staphylococcus aureus strains of sequence type ST398 which carried a type V SCCmec cassette (ST398-MRSA-V) gained considerable attention as they were found to colonise and cause infections in both, animals and humans with exposure to animal husbandry, especially swine farming. Strains of this type were first detected in The Netherlands, but are now also detected in other countries such as Germany, Belgium and Denmark.

Methods: Fifty-three independent strains obtained from pigs all over Germany on the basis of one strain per farm as well as one canine source of infection need to be considered. In order to identify ST398 MSSA, the 16S-23S interspace (IS) region lengths from all isolates were determined using specific primers in a PCR. Every S. aureus strain has 5 or 6 IS regions in its chromosome. The length of the individual regions vary within the chromosome, so when amplified and sorted by length using gel electrophoresis, each strain produces an unique pattern of bands.

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P1374 Evaluation of Clostridium difficile and methicillin-resistant Staphylococcus aureus contamination of retail chicken meat

J.S. Weese*, B. Acery, J. Rousseau, R. Reid-Smith (Guelph, CA)

Objectives: Concern has been raised about the potential for foodborne transmission of MRSA and C. difficile, yet there has been minimal investigation of these pathogens in chickens or retail chicken meat. This study evaluated the prevalence of C. difficile contamination of retail meat.

Methods: Chicken wings, legs and thighs were purchased from retail outlets in Ontario, Canada as part of the Canadian Integrated Program for Antimicrobial Resistance Surveillance. Samples rinsed in phosphate buffered saline, 1 ml of rinse was added to 9 ml enrichment broth (CDMN with sodium taurocholate), which was incubated for 24 h then inoculated onto CDMM agar. Isolates were characterised by ribotyping, PCR for detection of toxin A, B and CDT (binary toxin) genes and sequencing of the regulatory gene tcdC.

Results: C. difficile was isolated from 16/83 (19%) samples; 5/50 (16%) thighs, 6/28 (21%) wings, 2/5 (40%) legs (P = 0.40). All 12 tested isolates
were ribotype 078 (toxinotype V), possessed genes encoding toxins A, B and CDT, and had a deletion and mutation in tcdC. MRSA was not isolated from any sample.

**Discussion:** This study provides further evidence that *C. difficile* contamination of retail meat may be rather common. The finding of the potentially food-animal-associated ribotype 078 was somewhat surprising in light of a recent study reporting diversity of *C. difficile* types in chickens. The absence of MRSA is in contrast to recent studies of retail beef and pork in Canada, but is perhaps not surprising since MRSA has not yet been reported in poultry in North America. The clinical relevance of *C. difficile* contamination is unclear but further investigation of food in the epidemiology of community-associated *C. difficile* infection, as well as evaluation of sources of contamination and measures to reduce contamination, are indicated.

**Results:** The 100 ST398 isolates were non-typeable by SmaI-PFGE. They were negative for leukotoxins, exfoliatins and superantigen toxins. They carried only genes encoding haemolysins (hla, hlb, hlg ± hlg) and were of the agr quorum sensing system type I. Out-group and control isolates displayed different resistance patterns (tetracycline, oxacillin, erythromycin, and clindamycin being the most frequent resistances), and carried different SCCmec types (III, IVa, V or non-typeable), and spa types (mainly t011 and t034). The isolates were analyzed by Smal-PFGE, and screened for thirty seven genes involved in virulence and eight involved in tetracycline or erythromycin resistance by PCR.

**Conclusion:** The ST398 MRSA strains were lacking several virulence associated genes that were harboured by most of the HA-MRSA strains tested. These findings may reflect that this lineage has emerged from a peculiar ecological niche – possibly from another host than humans – and raise the issue of its colonising and infecting capacity for humans.

**Results:** 196 genes (49%) displayed variable results among the 25 strains tested. Each MLST lineage presented a specific gene profile that was highly conserved between strains belonging to a common MLST CC. ST398 strains displayed very homogenous gene profiles (>92% of homology) despite their host diversity. This “ST398-specific” profile was characterised by the absence of several virulence-associated genes harboured by most of the HA-MRSA strains tested, such as genes encoding enterotoxins, proteases (spl operon, sak), haemolysins (hlgB, hly) or adhesion factors (embp, ebpS, hlgB, mapW). The resistance gene profiles were less conserved within lineages than virulence-associated genes, except that possessing the tetM gene was a common characteristic of all ST398 strains.

**Conclusion:** The ST398 MRSA strains were lacking several virulence associated genes that were harboured by most of the HA-MRSA strains tested. These findings may reflect that this lineage has emerged from a peculiar ecological niche – possibly from another host than humans – and raise the issue of its colonising and infecting capacity for humans.

**Results:** The seven human findings, with no apparent connections between patients, were ribotype 078 (toxinotype V), possessed genes encoding toxins A, B and CDT, and had a deletion and mutation in tcdC. MRSA was not isolated from any sample.

**Discussion:** This study provides further evidence that *C. difficile* contamination of retail meat may be rather common. The finding of the potentially food-animal-associated ribotype 078 was somewhat surprising in light of a recent study reporting diversity of *C. difficile* types in chickens. The absence of MRSA is in contrast to recent studies of retail beef and pork in Canada, but is perhaps not surprising since MRSA has not yet been reported in poultry in North America. The clinical relevance of *C. difficile* contamination is unclear but further investigation of food in the epidemiology of community-associated *C. difficile* infection, as well as evaluation of sources of contamination and measures to reduce contamination, are indicated.
First infections by methicillin-resistant *Staphylococcus aureus* sequence type 398 in Spain

C. Potel, M. Álvarez-Fernández*, L. Constenla, V. Barbeito, P. Álvarez-garcía (Vigo, Pontevedra, ES)

**Objectives:** An emerging ST398 MRSA clone producing infections in humans has been detected in France, Netherlands, and Denmark. Recent Dutch studies indicate that it is widely distributed in farm animals particularly in pigs and may give rise to infection in humans.

In this study we present the three first cases of MRSA infections in Spain, which with all probability were acquired from contact with animals.

**Methods:** Three MRSA strains resistant to tetracycline were isolated in the Northwest of Spain (Pontevedra Province) in 2006. The isolates were studied by analysis of restriction fragment length polymorphism of the coagulase gene patterns (RFLP). All the strains were analyzed by multilocus sequence typing (MLST). The staphylococcal chromosome cassette (SCC) mec and the accessory gene regulator (agr) types were determined by multiplex and duplex PCR respectively. The presence of Panton-Valentine leukocidin-encoding (PVL) genes were identified by PCR.

Clinical and demographic data from the three patients were obtained.

**Results:** The three MRSA strains were identical by RFLP and different from the known local epidemic clonal lineages (ST5, ST36, ST125). The strains were ST398, SCCmeec-V, agr-1 and PVL genes negative. The prevalence of this clone was 1.8% (3 ST398 strains from a total of 168 MRSA isolated in 2006). The average age of the three patients was 75 years old. Two patients owned pigs and the other a cattle. They had had some admission to the hospital in 12 months before the diagnosis. Two patients were diabetic and developed skin and soft-tissue infections, the third one had bronchitis.

**Conclusion:** Because of the rapid adaptability of the ST398 clone, we must be aware about the increase of the prevalence of this clone in Spanish hospitals as it has been reported in the Netherlands recently. To limit further spread, strict isolation precautions should be taken in the case of patients with MRSA isolates typically tetracycline resistant.

The Northwest of Spain is very rural and many families have their own small farms, therefore veterinary studies are warranted to know the prevalence of this emerging clone.

Microbiological characterisation of *Staphylococcus pseudintermedius* isolates from dogs

M. Faires, S. Gard, D. Ancoin, J.S. Weese* (Guelph, CA; Irvine, Santa Monica, US)

**Objectives:** In dogs, *Staphylococcus intermedius* has traditionally been regarded as the predominant pathogenic *Staphylococcus* species and a leading cause of skin and soft tissue infections. However, it has been recently reported that most isolates identified conventionally as *S. intermedius* are truly related species *S. pseudintermedius*. The objectives of this study were to determine the prevalence of *S. pseudintermedius* among isolates from infections from dogs that have been classified, phenotypically, as *S. intermedius* and determine the prevalence of selected virulence factors and methicillin-resistance of *S. pseudintermedius* isolates.

**Methods:** Isolates from various infections in dogs that were phenotypically identified as *S. intermedius* were collected. Isolates were molecularly identified by sequence analysis of the sodA gene. For all isolates identified as *S. pseudintermedius*, genes for exfoliative toxins A (ETA) and B (ETB), *S. intermedius* exfoliative toxin (SIET), toxic shock syndrome toxin – 1 (TSST-1), Panton-Valentine leukocidin (PVL) toxin, and methicillin-resistance (mecA) were investigated. Each mecA positive isolate was evaluated for susceptibility to oxacillin (1 μg) and cefoxitin (30 μg) using the disk diffusion method and the presence of the penicillin-binding protein 2a (PBP2a) using a latex agglutination test (LAT).

**Results:** 102 isolates phenotypically identified as *S. intermedius*, were analyzed. 88/102 (86.3%) were molecularly identified as *S. pseudintermedius*. None were identified as *S. intermedius*. The SIEt gene was detected in 60.2% (53/88) of *S. pseudintermedius* isolates. Genes for ETA, ETB, TSST-1, and PVL were not detected. The mecA gene was identified in 15.9% (14/88) isolates. 11/14 (78.6%) methicillin-resistant strains were phenotypically resistant to oxacillin and produced PBP2a. However, none were identified as resistant to cefoxitin.

**Conclusion:** The re-classification of a large proportion of *S. intermedius* isolates as *S. pseudintermedius* provides additional support to the hypothesis that *S. pseudintermedius* is the predominant pathogenic *Staphylococcus* species in dogs. The SIEt gene was common and its role in disease requires further study. The low rate of cefoxitin-resistance but high rate of oxacillin-resistance in methicillin-resistant strains is opposite
to that reported for *S. aureus* and must be considered when developing testing regimens for methicillin-resistant *S. pseudintermedius*.

**P1381**  An investigation of a methicillin-resistant *Staphylococcus aureus* outbreak in marine mammals

M. Faires, J.S. Weese* (Guelph, CA)

**Objective:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is emerging as an important cause of morbidity and mortality in animals and has been found in an impressive range of species. In 2006, MRSA was isolated from the blowhole of a captive dolphin suspected of having pneumonia. To determine the extent of MRSA colonisation among the marine mammals, an investigation was conducted.

**Methods:** Nasal swabs were collected from consenting personnel, blowhole swabs were collected from dolphins and killer whales, and nasal swabs were collected from walruses and seals. Selective culture for MRSA was performed and isolates were typed via pulsed field gel electrophoresis (PFGE) and spa typing.

**Results:** On initial sampling, in January 2007, MRSA was not isolated from personnel (0/22), killer whales (0/4), or seals (0/16) but was isolated from dolphins (2/6, 33.3%) and a walrus (1/6, 16.7%). Colonised animals were isolated, contact with colonised animals was restricted and all personnel were required to wear gloves and masks when handling colonised animals. Routine hand hygiene was emphasized. Antimicrobials were not used for decolonisation. Following these recommendations, follow-up testing for MRSA colonisation was performed on the dolphins and walruses throughout 2007 and 2008 (Table). Overall, MRSA was isolated on one or more occasions from 5 dolphins and 3 walruses. All isolates were indistinguishable on PFGE and were consistent with the Canadian epidemic MRSA 2 (USA 100) strain, spa type t002, clonal complex 5 human epidemic clone.

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of MRSA positive animals/Total number of animals tested (% MRSA positive animals)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Dolphins</td>
</tr>
<tr>
<td>February 2007</td>
<td>2/6 (33.3%)</td>
</tr>
<tr>
<td>April 2007</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>May 2007</td>
<td>2/3 (66.7%)</td>
</tr>
<tr>
<td>October 2007</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>May 2008</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>July 2008</td>
<td>0/5 (0%)</td>
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<tr>
<td>October 2008</td>
<td>0/5 (0%)</td>
</tr>
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</table>

**Conclusion:** This is the first report of MRSA colonisation in several marine mammals with apparent transmission between species. A human origin is suspected because of the clone that was isolated. Colonisation was eliminated without antimicrobials, as has been reported in many animal species, however long term (14 months) colonisation was present in one dolphin. This study shows the impressive ability of MRSA to infect diverse animal species and provides further evidence suggesting that interspecies transmission of human epidemic clones can occur between people and animals.

**P1383**  Emergence and differentiation of pig-associated methicillin-resistant *Staphylococcus aureus* CC398 in Austria

G. Zarfel*, A.J. Grisold, G. Feierl, E. Leitner, L. Masoud, U. Wagner-Eibel, A. Badura, E. Marth (Graz, AT)

**Objectives:** For many countries in Europe a new form of CA-MRSA is reported, associated with animals, particularly pigs. This MRSA forms the clonal complex (CC) 398. MRSA CC398 consists of 8 MLST types and a range of closely related protein A (spa) gene types (e.g. t011, t034, t108, t567, t1451). In this study we report the first occurrence and emergence of MRSA CC398 in Austria. Because MRSA CC398 is not typeable with PFGE, determination of genetic relatedness was performed using rep-PCR on the new DiversiLab system (BioMérieux).

**Methods:** A total of 585 non-duplicated MRSA strains, isolated from 2002 to 2008 at the Institute of Hygiene, Medical University Graz, Austria, were investigated. The isolates were characterised by standard laboratory methods and resistance patterns were determined according to the CLSI guidelines. MRSA isolates that were defined as CA-MRSA, following the CDC criteria, subsequently were spa-typed. Determination of the genetic relatedness of strains belonging to MRSA CC398 spa types was performed using rep-PCR on the new DiversiLab system.

**Results:** A total of 13 MRSA CC398 could be detected, with first detection of MRSA CC 398 in 2004. MRSA CC398 was then present in every following year, with one MRSA CC398 strain in 2004 up to four MRSA CC398 strains in 2008. Spa-typing identified 11 isolates belonging spa-type t011, one to spa-type t034 and one spa-type t1451. Resistance patterns of all MRSA CC398 isolates showed, beside the resistance to all β-lactam antibiotics, resistance to tetracycline only. Only the spa-type t034 isolate showed additional resistances to clindamycin, erythromycin, ciprofloxacin, and sulphonamides.

**Conclusion:** This is the first report of MRSA colonisation in marine mammals with apparent transmission between species. A human origin is suspected because of the clone that was isolated. Colonisation was eliminated without antimicrobials, as has been reported in many animal species, however long term (14 months) colonisation was present in one dolphin. This study shows the impressive ability of MRSA to infect diverse animal species and provides further evidence suggesting that interspecies transmission of human epidemic clones can occur between people and animals.

**P1382**  Methicillin-resistant *Staphylococcus aureus* colonisation of veterinary personnel at a surgical conference

L. Burstin, M. Faires, J.S. Weese* (Tampa, US; Guelph, CA)

**Objectives:** Some studies of veterinary personnel and other individuals with animal contact have reported high rates of MRSA colonisation, but further information about colonisation of different groups and evaluation of factors associated with colonisation is required to better understand zoonotic MRSA transmission and develop control programs. This study evaluated the prevalence of and risk factors for MRSA colonisation among attendees of a veterinary surgery specialty conference.

**Methods:** Nasal swabs were collected from volunteers at the 2008 conference of the American College of Veterinary Surgeons in San Diego, USA. Enrichment culture was performed and isolates were characterised using standard techniques.

**Results:** 341 individuals from 12 countries participated. MRSA was isolated from 59/341 (17.3%, exact 95% CI 13.4–21.7%) individuals; 53/308 (17%) veterinarians and 63/183 (34%) technicians (P = 0.81). In the multivariable model, contact with small ruminants in the preceding 30 days (OR 2.2, 95% CI 1.1–5.6, P = 0.032), having another person in their residence diagnosed with MRSA in the preceding year (OR 19.8, 95% CI 1.9–203, P = 0.012) and working in a clinic where there is a specific person in charge of the infection control program (OR 1.9, 95% CI 1.1–3.5, P = 0.035) were associated with colonisation. The most common MRSA strain was spa type t002 or related types, which were PVL negative and accounted for 32 (54%) isolates. 16 (27%) were spa type t064 or related and PVL negative. 8 (14%) were t018 or related and PVL negative. 2 (3.7%) were t379 and PVL negative. 1 (1.9%) was t008 and contained PVL genes, consistent with the USA300 clone. Most individuals carrying spa type t002 or related were from small animal practices, while most people harbouring t064 were from equine practice.

**Conclusion:** This is the first report of MRSA colonisation in several marine mammals with apparent transmission between species. A human origin is suspected because of the clone that was isolated. Colonisation was eliminated without antimicrobials, as has been reported in many animal species, however long term (14 months) colonisation was present in one dolphin. This study shows the impressive ability of MRSA to infect diverse animal species and provides further evidence suggesting that interspecies transmission of human epidemic clones can occur between people and animals.
All affected patients had had contact to animals, in 12 cases to pigs; one person had contact to horses only.

**Conclusion:** In this retrospective analysis MRSA CC398 could for the first time be identified in patients in Austria and is present in Austrian patients since 2004. MRSA CC398 could only be detected in patients with close contact to animals, particularly pigs. Even if there was only a small number of MRSA CC398 from 2002 to 2007 active surveillance is once more needed to monitor the spread of this new MRSA subtype. Using the semi-automated rep-PCR method on the DiversiLab system may help to determine genetic relatedness of this new subtype of MRSA.

**P1384** First methicillin-resistant *Staphylococcus aureus* of ST398 isolated in Poland from nasal carriery

N. Marszalek, J. Engelm, J. Zmudzki, A. Nowak, Z. Pejsak, W. Hryniewicz (Warsaw, Pulawy, PL)

**Objectives:** Pig farming is a risk factor for carriage of *S. aureus* sequence type 398 (ST398). This new lineage of *S. aureus* has emerged in many countries. The aim of our study was the screening of veterinarians in Poland for the occurrence of MRSA ST398.

**Methods:** Nasal swabs were taken from participants at a conference concerning pig health and farming. All mecA-positive isolates of *S. aureus* were characterised by PFGE using Smal restriction enzyme, MLST analysis, spa-typing, agr-typing and SCCmec typing. Additionally, the detection of genes encoding virulence factors, such as enterotoxins (sea-sec, seg-sei), exfoliative toxins (eta, etb), tst, lukS-PV/lukF was performed. Susceptibility testing according to CLSI and CA-FSM criteria was determined for the following antimicrobials: tetracycline, daptomycin, fusidic acid, erythromycin and clindamycin.

**Results:** Among 222 samples, 5 MRSA isolates (2.25%) were detected. Four of them (1.8%) were PFGE nontypeable, had ST398, spa-type t034 (n = 1) or t108 (n = 3), SCCmec type V, agr-type I and were negative for any toxins. All of these isolates were fully susceptible to daptomycin and fusidic acid but resistant to tetracycline, the antibiotic most frequently used in pig farming. All but one isolate presented the MLSB constitutive phenotype.

**Conclusion:** This report first described cases of nasal colonisation with MRSA ST398 of a healthy veterinary staff in Poland. These strains were genotypically similar to those MRSA ST398 observed in many countries in Europe, Asia and America. It shows that MRSA ST398 has become a more common, international problem.

**P1385** Antibiotic resistance in the food chain: characterisation of *S. aureus* isolated from milk and cheese in Italy

G. La Salandra*, E. Crisetti, C. Pedrarra, D. Chiocci, A. Parisi, G.V. Celano, G.L. Germinario, G. Mulia, G. Normanno (Foggia, Bari, Sassari, IT)

**Objectives:** Milk and dairy products are the foods most frequently implicated in staphylococcal food poisoning, since *Staphylococcus aureus* could be present in humans and in ruminants. The use of antibiotics in human medicine and in veterinary practices, could determine the selection of antibiotic-resistant clones of *S. aureus*, including methicillin-resistant *S. aureus* (MRSA). In this note are reported the results of the characterisation of 151 *S. aureus* isolates from milk and cheeses produced in Italy.

**Methods:** 151 strains of *S. aureus* isolates from raw milk (37) and cheese samples (114) during 2007 were studied. They were characterised in order to determine the staphylococcal enterotoxin(s) (SEs) production (SEA to SED) by reverse-passive latex agglutination, the antibiotic-resistance profile using the disc agar diffusion method on Mueller-Hinton, and the detection of the mecA gene by PCR. Furthermore, the ecological origin of the strains were determined by typing.

**Results:** Among the 37 milk isolates, 17 (45.9%) resulted resistant at least to two antibiotics tested and, among these, 15 (88.2%) resulted resistant to ampicillin and 7 (41.7%) showed multidrug resistance proprieties (MDR). The main SEs detected were SEA (56.7%), followed by SED (21.6%). The most frequent ecovars were the Non Host Specific biotype (NHS) (48.6%) followed by the Bovine biotype (5.4%). Among the 114 cheese isolates, 25 (21.9%) resulted resistant at least two antibiotics tested and among these, 17 (68%) were resistant to ampicillin; 4 strains (16%) were MDR. The main SEs detected were SEA (17%) followed by SEC (16.6%) and the most detected ecovars were the NHS (26.3%) followed by the Ovine (11.4%). No MRSA strains were detected.

**Conclusion:** The presence of enterotoxogenic strains of *S. aureus* in milk and cheeses produced in Italy represent a potential risk for consumers especially in the absence of strict hygienic and preventive measures to avoid SEs production in these foods. A remarkable level of resistance to several antibiotics such as ampicillin, tetracycline and erythromycin was found in the *S. aureus* strains analyzed in this survey: this finding may constitute an additional risk of foodborne infection hard to be treated. The ecological origin of the analyzed strains was primary NHS, but most strains derived from human and animal reservoir. These findings calls for improved hygiene in the primary production and handling of milk and cheeses.

**Antibacterial susceptibility**

**P1386** Antibiotic susceptibilities of 90 isolates of *Yersinia pestis* to 14 antimicrobial agents

T. Meka-Mechenko*, L. Nekrassova, G. Temiraliyeva, B. Atshabar, G. Kocakawa (Almaty, KZ)

**Objectives:** Ninety isolates of *Yersinia pestis* isolated in 1998–2008 were evaluated for their susceptibilities to 14 antibiotics by the agar dilution method. Today, the majority of plague cases are sporadic. The most *Y. pestis* are exquisitely susceptible to commonly administered antimicrobial agents. But in 1995, a multidrug-resistant strain of *Y. pestis* was isolated in Madagascar from a 16-year-old boy. Besides, *Y. pestis* is one of several agents possibly to be used as biological means in bioterrorism case. Therefore constant monitoring of sensitivity plague strains to antibacterial preparations is very important.

**Materials and Methods:** Ninety strains of *Y. pestis* were tested in the present investigation. These strains were isolated over 10-year period in Republic of Kazakhstan (1998–2008). The sources of strains isolation were: animal carcasses, humans, fleas. The MICs of 14 antimicrobial agents for *Y. pestis* were determined by disk-diffusion methods. The antimicrobial agents tested included: amoxicillin, imipenem, cefalotin, cefoxitin, aztreonam, ofloxacin, pefloxacin, ciprofloxacin, streptomycin, gentamicin, amikacin, tobramycin, doxycycline, chloramphenicol.

**Results:** Investigated strains were confirmed as *Y. pestis* by standard criteria and had typical properties. Determination of antibiotic susceptibility is obligatory tests of study plague strains in laboratory practice in Kazakhstan. All the isolates were susceptible to all investigated antibiotics. There are no resistant strains. And there was no resistant or intermediate strain to imipenem. Though in the literature are described such *Y. pestis* strains. *Y. pestis* remains susceptible to most antibiotics tested with a higher efficacy for fluoroquinolones, cephalosporins and aminoglycosides. All the strains tested were susceptible to the antibiotics recommended for post-exposure prophylaxis. However, further in vivo studies are needed for determining alternative antibiotic treatments in case of bioterrorist attack with strains resistant to recommended antibiotics.

**Conclusions:** All *Y. pestis* strains were found susceptible to antimicrobial agents traditionally recommended for the treatment of *Y. pestis* infections. All the isolates were susceptible to β-lactam antibiotics including imipenem, to fluoroquinolones, aminoglycosides and to doxycycline.

We have no detected resistant *Y. pestis* strains during 10-year of investigation.
**P1387** Intraphagocytic activity of ciprofloxacin against intracellular Brucella melitensis


**Introduction:** Brucella is a facultative intracellular pathogenic bacteria which is able to multiply inside phagocytic cells. Human brucellosis is characterised by its trend to chronicity and recurrence, even when the antibiotic treatment was correct. The quinolones usually shown a very good activity “in vitro” against Brucella and they are able to get a very high concentration inside the phagocytic cells. However, the treatment of human brucellosis using ciprofloxacin did not reach the expected results. This study aims to determine the intraphagocytic activity of ciprofloxacin and rifampin against Brucella.

**Material and Methods:** Polymorphonuclear leukocytes (PML) were harvested from heparinised human blood by dextran sedimentation and differential centrifugation on Ficoll-Hypaque gradient. Smooth Brucella melitensis 16 M were opsonised with specific human IgG anti-Brucella. Neutrophils were coincubated with smooth opsonised B. melitensis 16M for 30 min at 37°C in a final volume of 1 ml HBSS plus 10% HNS. The extracellular bacteria were separated by differential centrifugation and the rest of extracellular bacteria were killed with streptomycin. The neutrophils were washed in antibiotic-free HBSS and then they were suspended in the same media or in HBSS containing various concentrations of antibiotics. The various systems were incubated for 30 and 60 min at 37°C after which the neutrophils were washed to remove antimicrobial agents. Intraphagocytic bacteria were released by the addition of 1 ml of distilled water and diluted and 100 μl of each dilution transferred to isosensitest agar plates. The plates were incubated at 37°C in a 5% CO2 atmosphere for 48 hour.

**Results:** Figure 1 shows the percentage of killing of intracellular phagocytosed Brucella in the presence of various concentrations of Ciprofloxacin. The bactericidal activity of Ciprofloxacin is dose-dependent and the curves are very similar to the Rifampin. There were not statistical differences between the bactericidal activity against phagocytosed Brucella at 30 minutes and 60 minutes.

The activity of Rifampin and Ciprofloxacin against phagocytosed Brucella is higher than the activity against the extracellular bacteria. This suggest that both antibiotics accumulates in the granulocytes, increasing their bactericidal activity.

![Figure 1. Effect of ciprofloxacin in the killing of phagocytosed Brucella.](image)

**P1388** In vitro activity of tigecycline, doxycycline, streptomycin, rifampicin, and ciprofloxacin against 70 strains of Brucella melitensis

F. Kibar, A. Yaman*, E. Zorluer, P. Ett, B. Kurtaran, A. Candecir (Adana, TR)

**Objectives:** Brucellosis is a common worldwide public health problem. The aim of this study was the investigation of the antibiotic susceptibility pattern of brucella isolates.

**Methods:** The susceptibilities of 70 Brucella melitensis isolates obtained from clinical samples were tested in vitro. MIC values of tigecycline, doxycycline, streptomycin, rifampicin, and ciprofloxacin detected by E-test method. For antibiotic susceptibility test, Mueller Hilton agar plates with 5% sheep blood agar, and the E-test strips were used. The plates were incubated at 37°C in a 5% CO2 atmosphere for 48 hour.

**Results:** According to the MIC50 and MIC90, doxycycline was found to be the most active agent; followed by tigecycline, ciprofloxacin, streptomycin, rifampicin. All the isolates were susceptible to doxycycline, streptomycin and ciprofloxacin (except one strain). Rifampicin had the highest MIC50 and MIC90 values. For rifampicin, 33 (%47) of brucella strains had higher MICs than 1 mg/l.

**Conclusion:** Brucella isolates remain susceptible in vitro to most antibiotics (doxycycline, streptomycin and ciprofloxacin, except rifampicin) used for treatment of brucellosis. In vitro activity of a new antimicrobial agent, tigecycline was slightly lower than doxycycline.

Table. In vitro susceptibilities of Brucella melitensis isolates to five antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Range (mg/l)</th>
<th>MIC50 (mg/l)</th>
<th>MIC90 (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tigecycline</td>
<td>0.004–1</td>
<td>0.38</td>
<td>0.5</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.047–1.5</td>
<td>0.19</td>
<td>0.25</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.25–1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.5–32</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.125–32</td>
<td>0.38</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**P1389** Anti-Helicobacter pylori and anti-internalisation activities of Thai folk remedies used to treat gastric ailments

N. Chaichanawongsarng*, R. Vilaichone, S. Amornnyongjarean, Y. Poocoravan (Bangkok, TH)

**Helicobacter pylori**, an aetiologic agent of active chronic gastritis and peptic ulcer disease, is now considered to be as invasive enteropathogen. Invasion of gastric epithelium cells contribute to persistent infection and eradication failure due to the bacteria could escape from antibiotic treatment and immune defence mechanism. The aims of this study were to investigate the anti-Helicobacter pylori and anti-internalisation activities of nine Thai plant extracts used for gastric ailments in traditional medicine including Kaempferia parviflora, Allium sativum, Musa sapientum, Curcuma longa, Cymbopogon citratus, Centella asiatica, Andrographis paniculata, Aloe vera and Ocimum basilicum. The minimum inhibitory concentrations against 11 clinical isolates and 2 reference strains of H. pylori were examined using an agar dilution method. The level of internalisation against HEp-2 cells was determined by a conventional gentamicin internalisation assay. Only the methanolic extracts of Curcuma longa and Kaempferia parviflora exhibited significant antibacterial activities at MIC of 32 μg/ml and 64 μg/ml respectively. Four common medicinal plants including Curcuma longa, Kaempferia parviflora, Allium sativum and Musa sapientum were further tested for their anti-internalisation activities against H. pylori ATCC 43504 at 3, 6 and 12 h of incubation using the concentration equal to their MIC. All four plant extracts showed inhibitory effects on the invasion of H. pylori to HEp-2 cells except Curcuma longa enhanced the invasion at 6 and 12 h. Although, Allium sativum and Musa sapientum demonstrated marked anti-internalisation
activities, the high concentrations of the extracts may have cytotoxic effects. However, the use of medicinal plants still have potential benefit in H. pylori eradication and could be a useful choice to avoid the antibiotic resistance. Moreover, the anti-internalisation activities may be a new strategy to prevent H. pylori infection and improve the therapy.

In vitro activities of 22 antimicrobial agents against macrolide-resistant Campylobacter jejuni and Campylobacter coli isolates


Objectives: The increasing emergence of macrolide resistance among Campylobacter jejuni and Campylobacter coli will complicate the treatment of bacterial gastroenteritis. The aim of this study was to compare the activities of various fluoroquinolones, macrolides and additional antimicrobial agents towards C. jejuni and C. coli, focusing special attention on macrolide-resistant strains. In addition, disk diffusion method was compared to agar dilution method in susceptibility testing.

Methods: We analyzed the in vitro activities of 21 anti-microbial agents by the standard agar plate dilution method against 232 C. jejuni and C. coli strains collected from Finnish patients between the years 2002 and 2005. Tigecycline susceptibilities were determined by the E-test method. Mutations causing macrolide resistance at Escherichia coli equivalent bases 2058 and 2059 of the 23S rRNA gene were analyzed by pyrosequencing.

Results: Of all 232 Campylobacter isolates, 19 were resistant to erythromycin (MIC ≥ 16 mg/L). Of the erythromycin-resistant Campylobacter strains, 18 (95%) were also ciprofloxacin-resistant. None of the resistant strains were resistant to imipenem or tigecyclin. Disk diffusion results were not always in line with the MIC results. 17 of the resistant strains were resistant to imipenem or tigecyclin. Tigecycline susceptibilities were determined by the E-test method. Mutations causing macrolide resistance at Escherichia coli equivalent bases 2058 and 2059 of the rRNA gene. None of the erythromycin-susceptible strains had point mutation at the E. coli equivalent base 2059 of the rRNA gene. None of the erythromycin-susceptible strains had point mutation at the E. coli equivalent bases 2058 and 2059 of the 23S rRNA gene.

Conclusions: 1. Of the antimicrobials studied tigecycline and sitafloxacin were in vitro most effective towards C. jejuni and C. coli, with low MIC values also for the macrolide-resistant strains. They might be good candidate for clinical trials on campylobacteriosis. 2. Of the 19 erythromycin-resistant C. jejuni strains, 18 (95%) were also ciprofloxacin-resistant and 15 (79%) had high MIC values for telithromycin (MIC >32 mg/L). None of these strains were resistant to imipenem or tigecyclin.

Antimicrobial susceptibility of genital Mycoplasma hominis and Ureaplasma urealyticum


Objectives: Ureaplasma urealyticum and Mycoplasma hominis are causally linked to urethritis, prostatitis, epididymitis, urethral syndrome, cervicitis and urolithiasis. Susceptibility testing of U. urealyticum and M. hominis is necessary, because it allows adequate antimicrobial treatment. The aim of this study was to determine the susceptibility of U. urealyticum and M. hominis to doxycycline, erythromycin, josamycin, ofloxacin, tetracycline, ciprofloxacin, azithromycin, clarithromycin, and pristinamycin.

Methods: This study included U. urealyticum and M. hominis strains with 10,000 colony-forming units detected in female and male patients. Clinical specimens examined were urethral and endocervical swabs. Detection, quantification and antimicrobial susceptibility testing of U. urealyticum and M. hominis in clinical specimens were performed by MYCOPLASMA IST 2 test (BioMerieux SA). In study period between April 2008 and December 2008, susceptibility testing was performed in 80 U. urealyticum and 5 M. hominis strains.

Results: Antimicrobial susceptibility testing results of U. urealyticum and M. hominis were shown on the Table 1 and Table 2. Both U. urealyticum (100%) and M. hominis (100%) were the most sensitive to josamycin, and the most resistant to ciprofloxacin (U. urealyticum 56.2%) and erythromycin, clarithromycin (M. hominis 80%).

Conclusion: Both U. urealyticum and M. hominis indicated high rates of susceptibility to doxycycline (99% and 100% respectively). We conclude that doxycycline may be used in empirical treatment of U. urealyticum and M. hominis genital infections.

Table 1: Antimicrobial susceptibility testing of U. urealyticum (n: 80)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>S, n (%)</th>
<th>I, n (%)</th>
<th>R, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline</td>
<td>79 (98.8)</td>
<td>0</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>68 (85)</td>
<td>6 (7.5)</td>
<td>6 (7.5)</td>
</tr>
<tr>
<td>Josamycin</td>
<td>80 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>34 (42.5)</td>
<td>28 (35)</td>
<td>18 (22.5)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>77 (96.3)</td>
<td>0</td>
<td>3 (3.7)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>19 (23.8)</td>
<td>16 (20)</td>
<td>45 (56.2)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>68 (85)</td>
<td>6 (7.5)</td>
<td>6 (7.5)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>70 (87.5)</td>
<td>3 (3.8)</td>
<td>8 (7.7)</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>79 (98.8)</td>
<td>1 (1.2)</td>
<td>0</td>
</tr>
</tbody>
</table>

S: susceptible, I: intermediate, R: resistant.

Table 2: Antimicrobial susceptibility testing of M. hominis (n: 5)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>S, n (%)</th>
<th>I, n (%)</th>
<th>R, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1 (20)</td>
<td>0</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Josamycin</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>4 (80)</td>
<td>0</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4 (80)</td>
<td>0</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3 (60)</td>
<td>1 (20)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>1 (20)</td>
<td>0</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

S: susceptible, I: intermediate, R: resistant.

Antibiotic susceptibilities of genital mycoplasmas in male patients with urethritis

M. Hua*, N.M. Luk, WH. Lam, M. Eastel, C. Fu, C.Y. Chan, K.K. Lo (Shatin, Hong Kong, HK)

Objectives: To determine the antibiotic susceptibility profile of Mycoplasma hominis and Ureaplasma urealyticum in male patients with urethritis.

Methods: During a nine-month period, male patients presented with urethritis were recruited. First-void urine specimens were collected. Microbial growth and susceptibilities results were determined by the use of Mycoplasma IST2 kit.

Results: A total of 523 patients were recruited. Ureaplasma urealyticum was isolated in 69 (13.2%) patients, Mycoplasma hominis was found in 15 (2.9%) of them. No patient exhibited co-existence of these two bacteria. For Ureaplasma urealyticum isolates, 62 (89.9%) and 42 (60.9%) of them were resistant to ciprofloxacin and ofloxacin respectively. Resistance against azithromycin, clarithromycin, erythromycin, josamycin and pristinamycin were 5.8%, 2.9%, 5.8%, 2.9% and 2.9% respectively. Resistance against azithromycin, clarithromycin, erythromycin, josamycin and pristinamycin were 66.7%, 100%, 0% and 0% respectively. No resistance was observed against doxycycline.
Conclusion: Fluoroquinolones resistance was very high among Mycoplasma hominis and Ureaplasma urealyticum isolates. Macrolides resistance was low in Ureaplasma urealyticum and Mycoplasma hominis, which are intrinsically resistant to many macrolides, only josamycin and the streptogramin pristinamycin were active. Doxycycline was active against both organisms. These results highlighted the emergence of antibiotic resistance in genital mycoplasmas. Thus, the treatment of which should be guided by antibiotic susceptibility testing and local antibiotic resistance pattern.

**P1393** Activity of linopristin/flopristin (NXL 103) against Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus

P. McGhee, G. Lin, G. Pankuch, A. Bryskier, P.C. Appelbaum* (Hershey, US; Romainville, FR)

**Background:** Lower respiratory tract infections apart from being caused principally by Streptococcus pneumoniae and Haemophilus influenzae, are increasingly being caused by meticillin-resistant Staphylococcus aureus (MRSA), and future empiric therapy for this infection must take all resistance phenotypes of all 3 bacterial species into consideration. Linopristin/flopristin (NXL 103) is an experimental oral streptogramin, being in a ratio of a 30:70 combination of linopristin and flopristin. This study examined the in vitro activity of linopristin/flopristin compared to a spectrum of other agents against 261 S. pneumoniae, 150 H. influenzae, and 200 MRSA strains. The pneumococci comprised 86 penicillin susceptible, 79 mec(A), 1 erm(B) and mec(A), 19 L4 and 3 23S rRNA]. The 150 H. influenzae (146 uypeutable, 4 serotype b), included 50 [β-lactamase negative, 79 [β-lactamase positive, and 21 BLNAR organisms. Two hundred MRSA included 128 community-acquired and isolated from sites throughout the US, and 40 were hospital-acquired; strains also comprised 2 hetero-vancomycin intermediate (hVISA), 25 VISA and 5 vancomycin-resistant (VRSA).

**Methods:** For pneumococci and MRSA, agar dilution using Mueller-Hinton + 5% added sheep blood for pneumococci was used, and microdilution using commercially prepared trays containing freshly prepared Haemophilus Test Medium was used for H. influenzae.

**Results:** Against pneumococci, both components of linopristin/flopristin were inactive, but synergy was observed when both components were combined, with MIC50 and MIC90 values of 0.12–0.5 mg/L and 0.25–0.5 mg/L in the various β-lactam and macrolide resistance phenotypic groups. By contrast, against H. influenzae linopristin was inactive and all activity resided in flopristin, whose MICs were the same as those seen with the combination: an MIC50 of 0.25 mg/L and MIC90 of 0.5–1 mg/L for all resistance phenotypes. Against MRSA strains linopristin/flopristin yielded MICs of 0.125–0.5 mg/L (MIC50 and MIC90 values both 0.25 mg/L) amongst vancomycin susceptible strains and 0.06–2 mg/L (MIC50 0.5 mg/L, MIC90 1 mg/L) amongst vancomycin non-susceptible strains.

**Conclusion:** Linopristin-flopristin (NXL103) was very potent against all strains of S. pneumoniae, H. influenzae, and MRSA tested, irrespective of resistance phenotype.

**P1394** A study on bacteria isolated from intra-abdominal infections in Japan and their antimicrobial susceptibility


**Objectives:** To obtain information for empiric therapy, define bacteria, especially obligate bacteria isolated from relatively recent cases of intra-abdominal infections in Japan and susceptibility patterns of those isolates.

**Methods:** Seventy-eight specimens were collected into anaerobic transport medium (selective and non-selective) in an anaerobic chamber. Anaerobic culture continued for 1 week. Identification of isolates was done principally using biochemical method. Molecular-biological method was also used in some cases. The minimum inhibitory concentrations were determined by agar dilution method according to the CLSI guideline.

**Results:** A total of 208 anaerobes and 138 aerobes were isolated from 68 culture-positive specimens. Major anaerobes were Bacteroides fragilis group, Gram-positive cocci, Fusobacterium spp., and spore (−) Gram-positive rods. Dialister invisus, Desulfovibrio spp, Synergistes spp., were included in minor Gram-negative anaerobes. Major aerobes were Enterobacteriaceae, Enterococcus spp. and Staphylococcus spp. Staphylococcus spp. was significant in post-operative infection. Carbapenemens and β-lactamase inhibitor (BLI)β-lactam (BL) kept potent activity to most of the isolated bacteria except for aerobic Gram-positive cocci. Susceptibility (%) to clindamycin based on CLSI breakpoint were 67% in B. fragilis, 25% in other species of B. fragilis group, and 78 to 100% in other Gram-negative and spore (−) Gram-positive anaerobes.

**Conclusion:** Carbapenemens and BLI/BL keep potent activity to most of isolates from intra-abdominal infection. Decrease of susceptibility rate in B. fragilis group species except B. fragilis is significant.

**P1395** Prevalence of quinolone susceptible Pseudomonas aeruginosa and Staphylococcus aureus in delayed-healing diabetic foot ulcers in Ekpoma, Nigeria

E. Agwa*, J. Ihongbe, N. Inyang (Ishaka,UG; Ekpoma,NG)

**Aim:** To investigate the prevalence and antibiogram of Pseudomonas aeruginosa and Staphylococcus aureus from delayed-healing foot ulcers of diabetic patients in Ekpoma.

**Methods:** Using standard aspecf microbiological methods, 220 delayed-healing diabetic-foot ulcer samples were analyzed for bacteria isolation, identification and susceptibility test. Chi-square (α=0.01) was used to test the statistical significance of data obtained.

**Results:** Out of 220 samples analyzed, 82.3% were infected (41.8% P. aeruginosa, 30.0% S. aureus and 10.5% co-infection of P. aeruginosa and S. aureus). There was statistically significant (p < 0.01) association between P. aeruginosa and S. aureus in the population studied. Among the quinolones tested, S. aureus and P. aeruginosa showed the highest (74.2% and 71.3%) and lowest (38.2% and 34.8%) susceptibilities to Levofoxacin and Sparfloxacin respectively. P. aeruginosa was also: 68.7% susceptible to rifampicin; 53.0% to erythromycin, 52.2% to vancomycin; 36.3% to ceftriazone; 36.5% to cefoxitin; and 32.2% to oxacillin. S. aureus was: 51.7% susceptible to rifampicin, 37.1% to cefoxitin; 33.7% to ceftriazone; 28.1% to vancomycin and 25.8% to oxacillin.

**Conclusion:** Delayed-healing diabetic foot ulcers in Ekpoma are colonised by levofloxacin and sparfloxacin susceptible Pseudomonas aeruginosa and Staphylococcus aureus. Surveillance with improved diagnostic facilities, surgical and biosurgical debridement of nonviable tissue, to halt progression of infection is recommended.

**P1396** Whether tablets for mouth disinfection possess antibacterial activity required by European Standard EN 1640:2006

S. Tyski*, E. Mikiciuk, E. Bocian (Warsaw, PL)

**Objectives:** Several medicinal or cosmetic products present on the market in Poland as well as in other countries, should act as oral antiseptics. It is claimed, that these drugs or cosmetics in tablets form possess antibacterial and sometimes anti-inflammatory activity. Recently, several European Standards have been created by European Standardisation Committee for establishing, whether an antiseptic or disinfectant has or does not have an antimicrobial activity under the laboratory conditions. The aim of the study was to analyze bactericidal activity of selected compressed lozenge (chewable tablets) or oral tablets for mouth disinfection and eradication of oral infections.
Corynebacterium macginleyi: susceptibility to 7 usual ophthalmic antibiotics

Á. Somodevilla*, D. Domingo, M.C. Martínez, T. Alarcón, M. López-Brea (Madrid, ES)

Objectives: Clinical importance of Corynebacterium macginleyi isolated from conjunctival infections has been recently recognized. The aim of this study was to evaluate the susceptibility of 23 Corynebacterium macginleyi isolates obtained from conjunctival samples (November 2006 to June 2008) to 7 usual ophthalmic antibiotics: penicillin, gentamicin, ciprofloxacin, tetracycline, vancomycin, rifampicin and linezolid.

Methods: Conjunctival swabs obtained from symptomatic patients were cultured onto blood and chocolate agar and incubated in aerobic and microaerophilic environment, respectively. Plates were incubated for two contact times (15 min and 1 h) and bacterial suspensions density of 1.5–5×10⁸ cfu/ml were applied. Investigated tablets were suspended in water (1 tablet/5 ml). This method was validated and the suitability of neutraliser in the assay was evaluated.

Results: Only 3 preparations: Neo-angin, Orofar and Strepsils fully comply with EN 1040 criteria for antiseptics. Required reduction of cells count (1 to 3 strains) was achieved but after 1 h contact. It was estimated that other investigated preparations completely do not comply with EN 1040:2006.

Conclusion: The products for clinical use should be evaluated according to normalised criteria, e.g. EN and these recommendations should ensure effective bacterial infection treatment. Preparations without required antibacterial – antiseptic activity should be excluded from the market.

P1397 In vitro activity of co-trimoxazole and comparators against Gram-negative and positive blood stream pathogens

K. Bowker*, A. Noel, S. Tomasselli, A.P. MacGowan (Bristol, UK)

Objectives: Co-trimoxazole (Co-t) has been reported to have a low risk of Clostridium difficile associated diarrhoea (CDAD). It is therefore of potential value as a prophylactic and therapeutic agent as part of an infection control strategy to reduce the incidence of CDAD. However, there is little recent data on its in vitro activity against key bacterial pathogens. In this study, we compared the in vitro activity of Co-t to comparators against Gram negative and positive blood stream infection (BSI) pathogens isolated in our hospital in 2006–07.

Methods: MICs were performed using CLSI methods against 725 BSI pathogens. Susceptibility was determined using EUCAST clinical breakpoints. For Gram negatives, the comparators were ampicillin (AMP), co-amoxiclav (CoA), cefuroxime (CXM), ceftriaxone (CTRI), ciprofloxacin (CIP), gentamicin (GEN) and ertapenem (ERT). For Gram positives, the comparators were erythromycin (ERY), vancomycin (VAN), ciprofloxacin (CIP), fusidic acid (FUS), penicillin (P), daptomycin (DAP), linezolid (LIN). Results: The proportion of Enterobacteriacea susceptible to Co-t is similar to CoA and CXM but inferior to CRTI, GEN and ERT. Co-t is active against most Gram positive pathogens excepting Enterococci.

Conclusion: Co-t demonstrated broad spectrum activity against Gram negative and Gram positive pathogens, including MRSA. Co-t may represent a useful formulary alternative to fluoroquinolones, cephalosporins and CoA in hospitals with high rates of CDAD.

P1398 In vitro activity of co-trimoxazole and comparators against Gram-positive and negative blood stream pathogens

W.T. Wu*, P.C. Chiang, T.L. Wu, H.C. Lai (Taoyuan Hsien, TW)

Objectives: Mycobacterium marinum belongs to Runyon class I mycobacterium. It causes soft tissue infection, bone and joints infections, and even disseminated infection. We encountered a few of failed cases. Susceptibility testing is important for the management of patients with tuberculosis and those with disease caused by certain nontuberculous mycobacteria. In this study, the minimal inhibitory concentrations of these isolated strains will be analysed by microdilution method.

Methods: Bacterial strains: Twenty five consecutive isolates were obtained from the Division of Bacteriology, Department of Clinical
Susceptibility tests: We used 96 wells plates for microdilution methods of susceptibility tests described by Wallace et al. We made bacterial 7H9 broth equal to 0.5 McFarland turbidity, then diluted to 1:1000, and delivered 0.01 ml into each well containing 0.1 ml broth. Each antimicrobial agent was added by twofold dilution by sequence.

Breakpoints: RIF \( \leq 1 \) mg per L; EMB \( \leq 5 \) mg per L; CLR \( \leq 16 \) mg per L; DOX \( \geq 4 \) mg per L; SMX \( \leq 32 \) mg per L; AMK \( \leq 32 \) mg per L; LZD \( \leq 8 \) mg per L.

Results: Among 25 isolates, they are all susceptible to EMB, CLR, AMK, and LZD. Most of them are susceptible to RIF, and SMX. The least active agent is DOX.

Table. In vitro susceptibilities of 25 Mycobacterium marinum isolates.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
<th>% Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>RIF</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>EMB</td>
<td>0.5</td>
<td>0.4–2.5</td>
</tr>
<tr>
<td>CLR</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>DOX</td>
<td>10</td>
<td>32–32</td>
</tr>
<tr>
<td>SMX</td>
<td>4</td>
<td>64–0.64</td>
</tr>
<tr>
<td>AMK</td>
<td>4</td>
<td>16–1–16</td>
</tr>
<tr>
<td>LZD</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Conclusion: M. marinum is an important microorganism causing aquatic animals-related infections in Taiwan. Doxycycline is not a good antimicrobial agent for treating such infections. Both rifampin and ethambutol remain the mainstay of regimens against M. marinum infection. Clarithromycin, linezolid, and amikacin are the alternative agents for treating M. marinum infections. For sulfamethoxazole, although high susceptible percentage was observed, as high as 20% (5/25) isolates (not shown in the table) are borderline susceptible (MIC = 16 mg/L); it may be not a good alternative agent for treating such infections.

P1401 Comparative in vitro activities of moxifloxacin and 6 other antimicrobial agents causing intraabdominal infection: results from the PRISMA study

H. Seifert* (Cologne, DE)

Objectives: Moxifloxacin is a fourth generation fluoroquinolone with bactericidal activity against both Gram-positive and Gram-negative aerobic and anaerobic bacteria, including those involved in intraabdominal infections (IAI). The Prospective In Vitro Study to Determine the Activity of Moxifloxacin against Isolates from Patients with Abdominal Infection (PRISMA) compares the susceptibilities of common pathogens causing serious IAI in hospitalised patients.

Methods: 3,240 aerobic bacterial isolates (including Citrobacter spp., n = 186; Enterobacter spp., 298; Enterococcus faecalis, 385; E. faecium, 291; E. coli, 731; Klebsiella spp., 400; Morganella morganii, 97; Proteus mirabilis, 200; P. vulgaris, 96; Pseudomonas aeruginosa, 264; Serratia spp., 57; and Staphylococcus aureus, 235) were collected from 32 centres (including 24 university hospitals) in Germany in 2007. MICs were determined at each centre using microbroth dilution for the following antimicrobials: ampicillin/sublactam (AMP/SUL); piperacillin/tazobactam (PIP/TAZ); ceftaxime (CTX); etarpenem (ERT); meropenem (MER); levofloxacin (LEV); and moxifloxacin (MOX). EUCAST guidelines were used for interpretation.

Results expressed as MIC50's and MIC90's (mg/L) are listed in the Table. Moxifloxacin showed good activity against common aerobic bacterial isolates causing serious IAI. Compared to levofloxacin, MICs for moxifloxacin were one dilution higher/lower for Gram-negative and Gram-positive isolates, respectively. Compared to AMP/SUL, PIP/TAZ and CTX, the antimicrobial activity of the two fluoroquinolones was higher against all Gram-negative species with the exception of E. coli. Ertarpenem and meropenem showed the highest in vitro activity against most bacterial species obtained from IAI.

P1402 In vitro susceptibilities of toxigenic Clostridium difficile strains to 10 antimicrobial agents

D Velissari, M Martsoukou, N Skarmoutsou, V Lapis, C Sarkatzidi, E Papafragas, M Kanelloupolou* (Athens, GR)

Objectives: Clostridium difficile (CD) was identified as the major cause of nosocomial diarrhoea and pseudomembranous colitis. Recently an increased number of CD community associated diarrhoea have been reported. The aim of this study was to determine the in vitro susceptibilities of 10 antimicrobial agents against toxigenic C. difficile strains isolated from adult inpatients attending a tertiary hospital during 2007–2008.

Material- Methods: A total number of 52 clinical strains isolated on Cycloserine Cefoxitin Fructose Agar, CCFA (Oxoid, Hampshire, England) were tested. The detection of C. difficile toxins was performed by the Rapid Enzyme Immunomossay for Toxins A+B (Immunocard, Meridian Bioscience Inc. Cincinatti Ohio). The susceptibilities to the antimicrobial agents were investigated by E-test (AB Biodisk Solna, England).
Tuberculosis diagnosis

**P1404** Accuracy of QuantiFERON-TB Gold test versus Tuberculin Skin test to detect latent tuberculosis infection in HIV-positive individuals in Iran


**Background:** Despite it is widely used for Latent Tuberculosis Infection (LTBI) detection and other TB clinical conditions, Tuberculin Skin Test (TST) bears several limitations. Because of considerable rates of false positive and false negative results obtained by TST, several methods have been introduced to be used interchangeably for detecting LTBI instead of TST. QuantiFERON-TB Gold Test (QFT) is a laboratory method which has recently attracted much attention and is said to be more specific than TST to identify LTBI. In this study we have attempted to identify QFT accuracy in detecting LTBI in HIV infected patients.

**Methods:** This cross-sectional study is conducted in a HIV clinic in Tehran in 2007. Totally, 50 HIV positive patients were recruited for the study. All patients had neither history of previous tuberculosis nor were currently affected by active TB. All cases had the history of BCG vaccination. Positive PPD was defined as ≥5 mm induration.

**Results:** Of total 50 HIV positive patients, 43 (86%) were males. Mean age of the cases was 38.4±7.2 (range=21–53). 36 (72%) had negative PPD test while 14 revealed positive PPDs. Of the positive PPD group, 12 had concomitant positive QFT, just one case was PPD positive but QFT negative, and QFT was indeterminate in one PPD positive case. Of 36 negative PPD tests 18 (50%) had negative QFTs, 8 (22%) had positive tests and 10 (28%) yielded indeterminate results. Agreement between PPD and QFT was 76.9% (κ = 0.54, 95% CI = 38.4–69.6, P value <0.001). However, there was no association between PPD results and CD4 counts.

**Conclusion:** Our study denotes that QuantiFERON-TB Gold Test renders more accurate results for LTBI detection in HIV infected patients compared to TST.

**P1405** Comparison of an interferon-gamma release assay with tuberculin skin test for the diagnosis of tuberculosis infection in a contact investigation

E. Perez-Escalonazo*, J. Gutierrez, E. Menor, J.C. Alados Arboledas, M.I. Lopez, S. de Tena, M.D. Lopez-Prieto (Jerez de la Frontera, ES)

**Objectives:** To evaluate the agreement of the QuantiFERON® TB Gold In Tube test (QFT) and the tuberculin skin test (TST) for the diagnosis of the tuberculosis infection (TBI) in a contact investigation (CI), and to establish the utility of QFT as a tool for the indication of the treatment of TBI.

**Methods:** We studied 337 immunocompetent persons with recent history of contact with tuberculosis patients and a different degree of exposition to the index case; average age was 39 years (SD: 18.6), 56.3% were women and 46% were vaccinated with BCG. All were screened with chest X-ray, TST, QFT (Cellestis, Australia) and risk factors were registered in a questionnaire. TST was performed by Mantoux method and a positive test was defined as an induration ≥5 mm. QTF was made according to the manufacturer specifications. We considered as vaccinated persons those presenting with a suggestive scar. CDC recommendations were followed for the interpretation of the QFT and the treatment of the TBI in contacts. Agreement between TST and QFT was assessed by the Cohen kappa coefficient.

**Results:** Agreement between the TST and the QFT was moderate among non-vaccinated population (74%, x0.48, CI 0.36–0.61) and poor among the vaccinated group (40%, x0.09, CI 0.03–0.15). Agreement was high for people with greater degree of exposition to index case, mainly in non-vaccinated group. TST(+)QFT(−) was the most frequently detected

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**Tuberculosis diagnosis**

**P1403** Comparative in vitro activities of moxifloxacin and 6 other antibiotics against anaerobic bacterial isolates causing intra-abdominal infection: results from the PRISMA study

H. Seifert* (Cologne, DE)

**Objectives:** Moxifloxacin is a fourth generation fluoroquinolone with bactericidal activity against both Gram-positive and Gram-negative aerobic and anaerobic bacteria, including those involved in intra-abdominal infections (IAI). The Prospective In Vitro Study to Determine the Activity of Moxifloxacin against Isolates from Patients with Abdominal Infection (PRISMA) compares the susceptibilities of common pathogens causing serious IAI in hospitalised patients.

**Methods:** 430 Gram-negative anaerobic bacterial isolates (including *Bacteroides distasonis*, *B. fragilis*, 238; *B. ovatus*, 10; *B. thetaiotaomicron*, 79; *B. uniformis*, 33; *B. culaitus*, 22; and *Prevotella spp.*, 21) were collected from 32 centres (including 24 university hospitals) in Germany in 2007. MICs were determined centrally using *B. fragilis* ATCC25285 as quality control strain. Results expressed as MIC50 and MIC90 (mg/L) are listed in the Table.

<table>
<thead>
<tr>
<th>Species</th>
<th>AMP/SUL</th>
<th>ERT</th>
<th>MER</th>
<th>LEV</th>
<th>MOX</th>
<th>CLI</th>
<th>NET</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. thetaiotaomicron</em></td>
<td>2/2</td>
<td>0.25/0.5</td>
<td>0.125/0.25</td>
<td>0.125/0.25</td>
<td>8/16</td>
<td>2/4</td>
<td>4/8</td>
</tr>
<tr>
<td><em>B. ovatus</em></td>
<td>0.25/0.5</td>
<td>0.125/0.25</td>
<td>0.125/0.25</td>
<td>8/16</td>
<td>2/4</td>
<td>4/8</td>
<td>2/2</td>
</tr>
<tr>
<td><em>B. fragilis</em></td>
<td>0.25/0.5</td>
<td>0.125/0.25</td>
<td>0.125/0.25</td>
<td>8/16</td>
<td>2/4</td>
<td>4/8</td>
<td>2/2</td>
</tr>
<tr>
<td><em>B. uniformis</em></td>
<td>0.25/0.5</td>
<td>0.125/0.25</td>
<td>0.125/0.25</td>
<td>8/16</td>
<td>2/4</td>
<td>4/8</td>
<td>2/2</td>
</tr>
<tr>
<td><em>B. culaitus</em></td>
<td>0.25/0.5</td>
<td>0.125/0.25</td>
<td>0.125/0.25</td>
<td>8/16</td>
<td>2/4</td>
<td>4/8</td>
<td>2/2</td>
</tr>
<tr>
<td><em>Prevotella spp.</em></td>
<td>0.125/0.5</td>
<td>0.06/0.25</td>
<td>0.125/0.25</td>
<td>8/16</td>
<td>2/4</td>
<td>4/8</td>
<td>2/2</td>
</tr>
</tbody>
</table>

**Conclusion:** Moxifloxacin showed good activity against most *Bacteroides* and *Prevotella* species involved in serious IAI. Resistance rates ranged between 10 and 20% (with the exception of *B. culaitus* with 59% of isolates being resistant). Clindamycin had only poor activity with 20–50% of *Bacteroides* isolates being resistant. Ampicillin/subactam and metronidazole were active against most isolates. Eratopenem and meropenem showed the highest in vitro activity against *Bacteroides* species obtained from IAI, however, 8% of *B. fragilis* isolates were resistant to the carabepenems.

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**Swedish studies**

According to the manufacturer's recommendations, *B. fragilis* ATCC 25285 was used as quality control strain.

**Results:** The MICs 50/MICs 90 as well as the MIC range (mg/L) were as follows: vancomycin 1/1.5 (0.5–3), metronidazole 0.125/0.25 (0.023–0.5), imipenem ⩾32/⩾32, meropenem 0.125–32, levofloxacin ⩾32/⩾32, moxifloxacin 1/⩾32 (0.75–⩾32), ofloxacin ⩾32/⩾32, rifampin ⩽0.002⩾0.002 (⩽0.002–⩾32), ticarcycline 0.064/0.25 (0.023–0.38), daptomycin 0.25/0.75 (0.038–6).

**Conclusions:** Our *C. difficile* strains were sensitive to vancomycin and metronidazole which constitute the first choice of treatment for the CD associated disease (CDAD). High resistance rates to the quinolones levofloxacin and ofloxacin were observed. Imipenem was inactive against the examined strains. Rifampin, daptomycin and ticarcycline which showed an excellent in vitro activity, will probably offer an alternative therapeutic option for CDAD.

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**Agreement between PPD and QFT was 76.9% (κ = 0.54, 95% CI = 38.4–69.6, P value <0.001). However, there was no association between PPD results and CD4 counts.**
discordant result, for vaccinated and non-vaccinated groups. A hundred eight non-vaccinated persons showed TST (+), from these 38 were negative for QFT. In the vaccinated group 132 showed TST (+), from these 92 were negative for QFT. The indication of TBI treatment made by TST and risk situation was modified in 52% of cases according to QFT test. We prescribed treatment of TBI by QFT in 8% of the contacts that did not have indication according to the TST.

**Conclusions:** Agreement between TST and QFT was moderate in non-vaccinated people and was improved in the subgroup with more than six hours/day of exposure to index case. Whereas, in vaccinated people the agreement was poor. The use of QFT allows to a better selection of infected individuals and to reduce the number of unnecessary treatments of the TBI, particularly in vaccinated population.

**Methods:** A total of 344 suspicious subjects to tuberculosis infection (>3 weeks productive cough or a chest-x-ray suggestive for tuberculosis) with or without contact with TB cases enrolled in this study. Gold standard for tuberculosis diagnosis was smear and culture. All subjects were tested using 5 tuberculosis units of purified protein derivative (PPD). The predictive value of induration was examined 48–72 hours after PPD administration.

**Results:** The prevalence of pulmonary tuberculosis was 20.05% in our cohort of study. Indurations of PPD ≥ 5 mm had a positive predictive value (PPV) 33% and negative predictive value (NPV) 91%, indurations ≥10 mm had 34% PPV and 85% NPV and indurations ≥15 mm had 40% PPV and 83% NPV.

**Conclusion:** TST does not consider being a valuable tool for the evaluation of household contacts and suspected cases of tuberculosis in populations with high BCG vaccination coverage. Concerning significance of TST in suspicious subjects to pulmonary tuberculosis in central province of Iran.

**Objective:** Tuberculin skin testing (TST) is used for the identification of individuals with infection by *Mycobacterium tuberculosis* and other non-tuberculous mycobacteria. However, its value for the evaluation of household contacts and suspected cases of tuberculosis in populations with high BCG vaccination coverage is controversial. We aimed to determine the prevalence of tuberculosis infection and the predictive value of TST in suspicious subjects to pulmonary tuberculosis in central province of Iran.

**Methods:** A cross-sectional study involving 54 patients with culture-confirmed pulmonary tuberculosis and 175 healthy individuals was carried out. The QFT assay was performed in blood samples according to the manufacturer's recommendations. The optimal cut-off level was recalculated from Receiver Operator Characteristic (ROC) analysis. Sensitivities and specificities of the QFT assay were calculated by using cut-off values of IFN-γ above 0.10, 0.20, 0.30, 0.35, 0.40, 0.50, 0.60 and 0.70 IU/ml.

**Results:** When the proposed by the manufacturer cut-off value of >0.35 IU/ml was used, sensitivity, specificity, positive and negative predictive value of the QFT assay was 83%, 67%, 44% and 93%, respectively. By using lower cut-off values (>0.10–0.30 IU/ml) a slight increase in sensitivity (2–8%) was associated with an even greater decrease in specificity (4–10%). On the contrary, ROC analysis indicated that by using higher cut-off values (>0.40–0.80 IU/ml), QFT specificity slightly improved (1–5%), but important loss of assay sensitivity was recorded (2–14%).

**Conclusion:** We evaluated the diagnostic accuracy of QFT assay with different cut-off values for the diagnosis of active pulmonary tuberculosis. The manufacturer’s recommended QFT cut-off value of >0.35 IU/ml of IFN-γ was found appropriate for the diagnosis of M. tuberculosis infection. The determination of new cut-off values for interferon-gamma (IFN-γ) detection as proposed by previous studies, might improve the assay’s sensitivity and specificity, but should be performed with caution in different populations.
were calculated and compared for QFT and TST tests. Agreement between QFT and TST was assessed by the kappa (κ) coefficient.

**Results:** A total of 229 patients were enrolled in the study. One hundred fifty-eight had a record regarding BCG vaccination. Forty-one (26%) of the 158 patients had been vaccinated. In total, the sensitivity and specificity of QFT, excluding those with indeterminate results, was 83% (45/54; 95% CI: 70–92%) and 67% (117/175; 95% CI: 59–74%), respectively. The sensitivity and specificity of TST was 74% (40/54; 95% CI: 60–85%) and 64% (112/175; 95% CI: 56–71%), respectively. The overall concordance between the QFT and TST tests was 72.1%, with a κ value of 0.435 (95% CI: 0.318–0.553). In the BCG-vaccinated subgroup, agreement between the two assays was 66%, with a κ value of 0.352 (95% CI: 0.105–0.599). The difference with the non-vaccinated subgroup (κ=0.452; 95% CI: 0.292–0.612) was considered to be not quite statistically significant (p=0.0576). Initial TST positive screening followed by a QFT positive result was found to have greater sensitivity and specificity in the non-vaccinated [sensitivity=22/28; 79% (95% CI: 59–92%); specificity=72/89; 81% (95% CI: 71–88%)] compared to the BCC-vaccinated subgroup [sensitivity=6/9; 67% (95% CI: 30–92%); specificity=24/32; 75% (95% CI: 57–89%)].

**Conclusion:** This study confirmed previous reports that QFT assay has higher sensitivity for detecting active TB compared to TST. An overall moderate agreement between TST and QFT was found. The difference in agreement between non-vaccinated and BCG-vaccinated subgroups could be attributed to TST influence by vaccination. In patients with active TB and no BCG-vaccination history, TST screening followed by subsequent QFT testing proved to present the highest sensitivity and specificity for TB diagnosis. Larger prospective studies are needed to confirm our results.

**P1410 Evaluation of peripheral blood mycobacterium tuberculosis PCR in tuberculosis patients**

A.R. Davoodi*, M. Haji Abdolbaghi, M. Rasooli Nejad, A. Tayyebi (Bandar Abbas, Tehran, IR)

**Background:** Tuberculosis is still one of the most important cause of mortality and morbidity in many countries and is second only to human Immunodeficiency virus as a cause of death worldwide resulting from a single infectious agent, and in 1993 the World Health Organization declared tuberculosis a global public health emergency. Conventional methods for the diagnosis of Mycobacterium tuberculosis infections are time consuming, for example culture needs 3–8 weeks to grow and there is a need for new methods for accurate and rapid diagnosis of tuberculosis. To determine the sensitivity of polymerase chain reaction (PCR) in peripheral blood mononuclear cells (PBMC), we have evaluated Mycobacterium tuberculosis DNA in peripheral blood samples with PCR technique in adult patients with new cases of pulmonary and extra–pulmonary tuberculosis.

**Material and Methods:** In a cross sectional study lasting 3 years (2004–2007), 3cc Citrated Blood samples were obtained from 190 patients with pulmonary and extra – pulmonary tuberculosis. DNA extraction by QIAGEN (commercial kit) and PCR with IS1081 Primer was performed. For prevention of cross contamination and reduction of false positive, all steps were performed under laminar hood.

**Findings:** 134 cases of pulmonary and 56 with extra-pulmonary tuberculosis were enrolled in this study. PCR was positive in 78 of 190 (41%) patients and negative in 112 of 190 (59%). The overall sensitivity and accuracy of the PCR assay was 50% for pulmonary and 25% for extra-pulmonary and 28.5% for disseminated tuberculosis.

**Conclusion:** The use of IS1081 primer MTB-PCR assay on PBMC may pose problems for the rapid diagnosis of tuberculosis with regard to low sensitivity. However, further studies are needed to confirm this technique as an alternative test for the diagnosis of tuberculosis.
Results: A total of 352 samples were processed successfully on both days such that the T-SPOT.TB assay results could be compared between fresh blood samples processed within 8 hours of collection and stored blood samples processed between 16 and 32 hours after collection. The overall agreement was 342/352 (97.2%; 95% CI = 95.1–98.7%) with a kappa value of 0.94. In samples that were taken from culture confirmed TB cases 42/43 (97.7%) were T-SPOT.TB +ve for both the fresh and the stored blood samples.

<table>
<thead>
<tr>
<th></th>
<th>&gt;16 hr +ve</th>
<th>&gt;16 hr −ve</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>&lt;8 hr +ve</td>
<td>144</td>
<td>5</td>
<td>149</td>
</tr>
<tr>
<td>&lt;8 hr −ve</td>
<td>5</td>
<td>198</td>
<td>203</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>203</td>
<td>352</td>
</tr>
</tbody>
</table>

Conclusion: The study demonstrates that processing of blood samples, with only a minor addition to the overall assay procedure, the day following collection is practicable and yields substantially equivalent performance in the T-SPOT.TB assay.

Objective: Mycobacterial culture of sputum (MCS) is time-consuming but remains a gold standard for laboratory diagnosis of pulmonary tuberculosis (PT). The use of the Amplified Mycobacterium tuberculosis Direct Test (MTD, Gen-Probe; San Diego, CA) for direct identification of mycobacterium tuberculosis complex (MTBC) in sputa may aid to early diagnosis of PT. The MTD in diagnosis of PT is well studied in Chinese. Methods: An observational study was conducted to evaluate the test performance of MTD in diagnosis of Chinese PT. All respiratory specimens submitted to MTD were tested together with acid-fast (AF) stain of sputa and MCS. The sediment of each specimen was prepared after digestion, decontamination and concentration by the NALC-NAOH method. The sediment was tested by MTD according to the manufacturer’s protocol. Results: A total of 218 respiratory specimens obtained from 208 Chinese patients were analyzed. The final diagnosis of PT was regarded as a gold standard. Area under Receiver Operator Characteristic (ROC) curves of MTD and MCS were compared for diagnostic accuracy of PT.

Results: A total of 218 respiratory specimens obtained from 208 Chinese patients were analyzed. The final diagnosis of PT was made in 118 (56.7%) patients. AF bacilli were found in 177 (81.2%) specimens. MTBC-positive rates of specimens were 109 (50%) and 91 (41.7%) detected by MTD and MCS, respectively. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MTD in diagnosis of PT were 83.1%, 93.6%, 94.5% and 80.7%, whereas they were 65.3%, 100%, 100% and 68.6% for MCS. The area under ROC curve (95% confidence interval) was 0.88 (0.84–0.93) and 0.87 (0.82–0.92) for MTD and MCS, respectively. Positive and negative likelihood ratios of MTD were 13.0 and 0.18, respectively.

Conclusion: The MTD was demonstrated to have a comparable diagnostic accuracy of PT as MCS, implicating its clinical usefulness in direct detection of MTBC in respiratory samples and improvement of early diagnosis of PT.
Experience with tigecycline in infections due to Mycobacterium abscessus complex and Mycobacterium chelonae


Objectives: Treatment of infection with rapidly growing mycobacteria (RGM) including Mycobacterium abscessus complex (M. abscessus, M. massiliense, M. bolletii) and M. chelonae is difficult, particularly among patients (pts) with M. abscessus complex lung disease, as RGM are resistant to most antibiotics. Tigecycline, a first-in-class expanded broad-spectrum glycylcycline antibiotic, was examined for efficacy in the treatment of RGM infections in a combined analysis of data obtained from three settings.

Methods: Clinical efficacy and safety data from pts with RGM infection were pooled and assessed from: (1) an open-label clinical trial of pts with RGM infection with resistant isolates (7 pts); (2) a multicentre, open-label trial of pts with multiple drug-resistant pathogens resistant to standard therapies (7 pts); (3) a single-patient, compassionate-use program for pts who had failed or were intolerant of other therapies (38 pts). Target tigecycline dosage was 50 to 100mg daily, in single or twice-daily doses, with treatment duration as clinically appropriate. Other antibiotics could be administered concomitantly.

Results: Among the 52 pts, the median (range) age was 32 (12–81) years. The lung was the site of infection in 36 (69.2%) pts; 38 (73.1%) had M. abscessus complex infection, 9 (17.3%) had M. chelonae infection, and 5 (9.6%) were infected with both organisms. Treatment discontinuation occurred in 29 (55.8%) pts, 16 (30.5%) of these due to adverse events (AEs), primarily vomiting and/or nausea (9/16). A total of 25 pts (48.1%) were considered clinically improved, 16 (30.8%) were considered clinical failures, and 11 (21.2%) were indeterminate. The most common AEs were nausea in 33 (63.5%), vomiting in 18 (34.6%), fever in 13 (25.0%), diarrhea in 12 (23.1%), and anemia and anorexia in 11 pts each (21.2%).

Conclusions: Clinical experience in 52 pts with RGM infection treated with tigecycline in combination with other antibiotics demonstrated clinical improvement in nearly 50% of pts and an acceptable safety profile in pts treated for up to 3 years.

On the frontline of the already established vaccines

S395

Serologic response to influenza vaccine in coronary artery disease patients: FLUVAC study


Background: Recent studies suggested influenza vaccine may reduce the risk of ischaemic events in coronary artery disease (CAD) patients but no data was available on serologic response in this group. We conducted a clinical trial to investigate the efficacy of 2007–08 influenza vaccine (FluVacc) in inducing serologic response in CAD patients (ClinicalTrials.gov - NCT00607217). We are now presenting the serologic response to the FluVacc antigens.

Methods: A trial was conducted from January to August 2007 and enrolled 204 study subjects (137 CAD and 67 healthy controls [HC]). Each enrolled participant received 0.5 cc intramuscular dose of trivalent anti-influenza vaccine. The 2007–2008 influenza vaccine consisted of three strains, nominated as Solomon Island/3/2006[H1N1], Wisconsin/67/2005[H3N2], and Malaysia/2506/2004[B]. Antibody (Ab) titers (haemagglutination inhibition) were measured just before and 1 month after vaccination. The proportion of protective Ab titers (i.e. >1/40), the serological response (i.e. 4-fold rise in titers) rates, and the magnitudes of change in titers were the main outcome measures. Angina severity (Seattle angina questionnaire – SAQ), coronary artery stenosis (modified Gensini), and cardiac ejection fraction (EF) were measured.

Results: Serologic response against H1N1 antigen was observed in 90 (65.7%) CAD patients and 39 (58.5%) HCs. CAD and HC groups were similar in all outcome measures for all antigens (Figure). SAQ, Gensini and EF score were not significantly correlated with the magnitude of change in any of the Ab titers in CAD patients. Valvular heart disease and lower baseline titers were independently associated with the magnitude of antibody titer increment against H1N1 antigen in CAD group. Multivitamin supplement was independently associated with better antibody response against H3N2 antigen. Multivariate analysis failed to recognize any independent factor associated with the magnitude of titer change against B antigen.

Conclusions: CAD and HC groups were not significantly different in serologic response and magnitude of change in antibody titers against each of the vaccine antigens. Severity of CAD does not have any significant impact on the magnitude of serologic response.

Table. Response measures to the antigens of 2007–2008 trivalent influenza vaccine

<table>
<thead>
<tr>
<th></th>
<th>CAD, n = 137</th>
<th>HC, n = 67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solomon Islands/3/2006 (H1N1)</td>
<td>Magnitude of change, × fold, median (IQR)</td>
<td>4 (14)</td>
</tr>
<tr>
<td></td>
<td>Serologic response (≥4-fold HI titer rise), n (%)</td>
<td>90 (65.7)</td>
</tr>
<tr>
<td>Wisconsin/67/2005 (H3N2)</td>
<td>Magnitude of change, × fold, median (IQR)</td>
<td>4 (4)</td>
</tr>
<tr>
<td></td>
<td>Serologic response (≥4-fold HI titer rise), n (%)</td>
<td>100 (73.0)</td>
</tr>
<tr>
<td>Malaysia/2506/2004</td>
<td>Magnitude of change, × fold, median (IQR)</td>
<td>4 (6)</td>
</tr>
<tr>
<td></td>
<td>Serologic response (≥4-fold HI titer rise), n (%)</td>
<td>78 (56.9)</td>
</tr>
</tbody>
</table>

The efficacy of influenza vaccination in reducing cardiovascular events in patients with coronary artery diseases: IVCAD study


Objectives: It is a matter of controversy whether influenza vaccine is effective in reducing the risk of ischaemic events in coronary artery disease (CAD) patients. Our clinical trial was conducted to investigate the efficacy of 2007–2008 influenza vaccine (FluVacc) in reducing adverse cardiac events in CAD patients.

Methods: A trial was conducted from January to August 2008 (ClinicalTrials.gov - NCT00607178) and enrolled 281 CAD patients. They were randomised to receive either FluVacc (Flu-influcav, n = 141) or placebo (CAD-Placebo, n = 140). Antibody titers against influenza vaccine subgroups were measured before and 1 month after vaccination and the results were reported separately. CAD patients were followed for 6 months and angina severity (Seattle angina questionnaire – SAQ) before and 6 months after vaccination, coronary artery stenosis score (modified Gensini), cardiac ejection fraction (EF), number of flu episodes
and cardiac adverse event (acute coronary syndrome [ACS], coronary revascularisation, or cardiovascular death) were recorded as outcome measures.

**Results:** 135 CAD-Placebo and 131 CAD-Influvac subjects completed the study. The CAD-Placebo group experienced influenza infection significantly more than the CAD-influvac group (P = 0.049). Two cardiovascular deaths happened in CAD-Influvac group which was comparable with one death in CAD-placebo group. None of the secondary endpoint (6 months) outcome measures were markedly different among the two groups when compared individually. However, when occurrence of at least one of the outcome measures in each subject was considered, CAD-placebo group had significantly higher cardiac adverse events (ACS, coronary revascularisation, or cardiovascular death) than their CAD-Influenza counterparts. Angina severity scores (SAQ) improved more in CAD-Influenza than in CAD-Placebo group.

**Conclusion:** Influenza vaccine reduces cardiac adverse events and improves SAQ score in patients with coronary artery diseases in 6 months follow up. Timely influenza vaccination is highly recommended in this group of patients.

**P1419 Long-term immunogenicity of influenza vaccine among the elderly adults**


**Background:** Concerning the high risk of serious complication from influenza, the elderly have been considered as the priority group of influenza vaccination. However, influenza vaccine-induced antibody had been thought to decline more rapidly in the elderly.

**Methods:** During 2007–2008 influenza seasons, this study was conducted to compare the long-term immunogenicity of inactivated trivalent influenza vaccine among the elderly compared to the healthy young adults. Study subjects were stratified into four groups: 18–49 aged healthy adults, 50–64 aged healthy adults, 50–64 aged adults with co-morbidities and ≥65 aged adults. Serum haemagglutinin inhibition (HI) antibody titers were determined against the recommended influenza strains A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2) and B/Malaysia/2506/2004, at pre-vaccination and 1, 6 and 12 months after vaccination.

**Results:** Of the 1,018 enrolled subjects, 716 (70.3%) were followed during 12 month period. Seroprotection (titer ≥ 40) rate at 1 month post-vaccination in 18–49 aged healthy adults, 50–64 aged healthy adults, 50–64 aged adults with co-morbidities and ≥65 aged adults were 89.4% vs. 90.3% vs. 87.4% vs. 79.8% for influenza A/H1N1 virus, 87.6% vs. 89.1% vs. 89.8% vs. 85.0% for A/H1N1virus, and 89.4% vs. 74.3% vs. 70.1% vs. 73.1% for influenza B virus. At six months later, compared to 1 month post-vaccination, seroprotection rates for all three strains declined significantly in ≥65 aged adults (p < 0.001), but still met the CHMP criteria: A/H1N1 (69.2%), A/H3N2 (77.9%) and B (64.4%). At 12 month after vaccination, seroprotection rates for all three strains declined far below the CHMP criteria irrespective of age and co-morbidities. Intriguingly, seroprotection rates were not influenced by pre-immunisation history, but good responders (pre-HI titer ≥40) showed superior long-term immunogenicity compared to the poor responders; even in the elderly aged ≥65 years, seroprotection rates were maintained ≥60% up to 12 month post-vaccination against all three strains.

**Conclusion:** Current influenza vaccine showed adequate immunogenicity for Korean adults including ≥65 year aged persons. However, in the elderly, long-term immunogenicity of current conventional influenza vaccination was marginally seroprotective. Strategy to improve the immunogenicity of influenza vaccine may need to be considered in ≥65 aged adults such as adjuvanted vaccine, high-dose vaccine, intradermal vaccine, etc.
Polymorphism of pertactin gene in circulating Bordetella pertussis strains in Serbia

G. Dukić*, T. Kallonen, A. Elomaa, T. Pljesa, M. Viganjovic-Krstacucevic, M. Scubic-Vlahovic, Q. He (Belgrade, RS; Turku, FI)

Objectives: Despite the routine use of pertussis vaccines, pertussis remains an important cause of disease among young infants, adolescents and adults. Pertactin (Prn), a 69 kDa outer membrane protein of Bordetella pertussis, induces protective immunity and is included in the acellular pertussis vaccines. Polymorphism in Prn gene of circulating B. pertussis strains has been found in many countries with long vaccination tradition, and today 12 allelic variants (prn1–12) have been reported. The allele prn1 or prn7 are present in most vaccine strains and predominated in the pre-vaccine era, and the allele prn2 is by far the most prevalent type in actual isolates in most of countries. However, little is known of polymorphism of Prn gene in B. pertussis isolates in Serbia where vaccination against pertussis has been used for 50 years (pertussis vaccination was started in this country in 1957). The aim of this study was to analyse and compare Prn types in the Serbian isolates collected since 1950s.

Methods: The analysis included genotyping of prn by sequencing and LightCycler PCR. A total of 56 clinical isolates together with four vaccine strains were tested. The clinical isolates were from 1953 to 1958 (n = 16) and from 1981 to 2000 (n = 40).

Results: The allele prn1 was predominant among the Serbian isolates, although the prn2 (5%), prn3 (10%) and prn11 (15%) occurred in some isolates over the period from 1981 to 2000. The alleles prn3 and prn11 were detected in 1981 and 1984, respectively. However, the allele prn2 was only found in two strains isolated in 2000. The current Serbian whole-cell vaccine has been used since 1985 and contains both prn1 (three strains: 1772/57, 2047/57 and 23/81) and prn2 (one strain: 8/84). Interestingly, the vaccine strain 8/84 was isolated in 1984.

Conclusions: The frequency of modern B. pertussis isolates with prn2 was significantly low in Serbia compared to that found in most of countries with long vaccination histories. In contrast to other European countries, the prevalence of strains with prn2 seemed to come late in Serbia. The real impact of the inclusion of prn2 strains in vaccines on the antigenic variation of Prn and occurrence of new Prn types in B. pertussis population should be further investigated.

Is BCG vaccine scar necessary for confirmation of immunity against Mycobacterium tuberculosis infection?

S. Sayyahfar*, A. Karimi, S.A. Fahimzad (Tehran, IR)

Objective: Comparison of Gamma Interferon response to human PPD in scar negative and scar positive BCG vaccinated children.

Methods: Between August 2006 and May 2007 a total of 236 patients aged 1 month to 168 months (mean 21 months) admitted to different wards of Mofeed children hospital and met the inclusion criteria were enrolled in a cross sectional study with sequential manner. Each patient was examined for BCG vaccine scar and then tested with both Tuberculin skin Test (TST) and human PPD based Interferon Gamma release assay (IGRA).

Results: From total of 236 patients, 15 (40% female, 60% male, 1-156 months, mean 42 months) who were scar positive, 100% were TST negative. In scar negative patients the IGRA was positive in 10 (66.7%), negative in 4 (26.7%) and indeterminate in 1 (6.7%) respectively. 221 cases (44% female, 56% male, 1-168 months, mean 21 months) were scar positive. 5% and 95% of scar positive cases were TST positive and negative respectively. In scar positive patients the IGRA result was positive in 110 (49.8%) negative in 85 (38.5%) and indeterminate in 26 (11.8%) respectively.

Conclusions: It seems that immunity against mycobacterium tuberculosis in scar negative children does not relate to scar formation and may be better evaluated with a more accurate tool such as IGRA than contenting with scar formation alone.

Meningococcal disease in Italy in the era of conjugate menC vaccination

P. Stefanelli*, C. Fazio, T. Sofia, A. Neri, P. Mastrantonio (Rome, IT)

Objectives: Invasive Meningococcal Disease (IMD) remains a life-threatening disease. To determine the change in epidemiological characteristics of IMD in Italy after the introduction of conjugate menC vaccine in 2005, analysis of the microbiological features of isolates and of the clinical characteristics of patients has been carried out.

Methods: MD cases from 2005 through 2008 were identified according to the National Meningococcal Surveillance System. Serogrouping, sero/subtype and susceptibility testing were performed on all the meningococci received at the National Reference laboratory at the Istituto Superiore di Sanità.

Results: In 2005 and 2008, IMD showed an incidence of 0.5 and 0.2 x 100,000 inhabitants, respectively. While the incidence due to serogroup B remained quite stable in the total population as well as in the 0–4 and 15–24 age ranges, IMD incidence due to serogroup C has decreased in the total population since 2005. In particular, the decrease was markedly significant among infants with an incidence of 0.46 and 0.5 per 100,000 inhabitants in 2006 and 2007 respectively, vs. 1.69 and 1.29, in 2004 and 2005. A less significant decrease, was found among adolescents and young adults. Information on the clinical presentation was registered for 88% of cases; outcome was known for 82%. Clinical manifestations and outcome of infections underlined more severe diseases associated with C:2a isolates, with an increase in septicaemia from 28% in 2005 to 70% in 2007. Conversely, septicaemia cases due to C:2b remained quite stable: 45% and 33%, respectively. In the same period, fatal cases due to C:2a meningococci increased, from 7% to 55%. All the examined strains were susceptible to rifampicin, ceftriaxone, ciprofloxacin and penicillin. However, 82% of C:2b showed a decreased susceptibility to penicillin.

Conclusions: The Italian setting, following the introduction of meningococcal C conjugate vaccine recommended by the Ministry of Health but applied according to different regional strategies, can provide insight on possible effects of vaccination not only in the decreasing incidence of the disease but also on the prevalent spreading of specific meningococcal types. Data from ongoing surveillance of IMD to evaluate long-term effects of vaccination and to monitor the disease burden, predominantly caused by serogroup B meningococci, will be further evaluated.

Prospective study assessing the tetanus immunisation status and its determinants among patients consulting for wound care at the emergency department of a non-university hospital

S. Fanello*, V. Delmas, J. Choukraou, Y. Lannehou (Angers, Le Mans, FR)

Tetanus is a severe acute toxo-infection, often lethal, caused by a neurotoxin produced by Clostridium tetani; it can be prevented by vaccination.
**Objective:** To assess the tetanus vaccination status and define its main causative factors; to compare the patient’s self-declared vaccination status with his actual immunisation cover.

**Methods:** Prospective study assessing the tetanus immunisation status based on reliable evidence (injection certificates or point of care quick serodiagnostic test) and its determinants among patients consulting for wound care at the Emergency Department of a non university Hospital.

**Results:** 1120 patients were included. 50% declared they had their boosters in time, 31% didn’t know their vaccination status, and 16% had some kind of vaccination certificate. As for actual coverage, it appeared that only 61% were immunised and 8% not immunised against tetanus, but for 30.5% it was unspecified (no document, no test). The cover decreased significantly with age. It was better for men.

**Conclusion:** According to current data, our findings confirm that only 61% of the population is immunised against tetanus, with a lack of coverage in particular for women and elderly. Therefore we have to change our practices to increase immunisation coverage and reduce the use of specific human immunoglobulins for tetanus prophylaxis.

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**P1428** Evaluation of anti-measles IgG antibody level in medical students 4 years after mass vaccination

N. Jonaidi Jafari*, M. Izadi, M. Kakaei, R. Ranjbar, M. Mohammad Jonaidi (Tehran, IR)

**Objectives:** Measles is a severe contagious disease that can be prevented by vaccination. In addition to prevaleance of measles in Iran, ministry of health and medical education has done a national wide vaccination against measles for all 5–25 years old population in 2004. Edmunston Zagreb vaccine was used. This study was done to evaluate anti measles IgG antibody in medical students 4 years after mass vaccination.

**Methods:** This is a cross-sectional study on 196 medical students of Baqiyatallah University of medical science. The chart contain age, history of vaccination in childhood, history of vaccination in 2004, history of measles, history of measles in family history of contact with measles case and history of fever in time of vaccination. IgG confirmed with ELISA test in 5 cc blood that taken from each cases. The IBL German kit was used.

**Results:** In this study total of case was man. With mean age of 23±5±3±01. History of vaccination in childhood in 194 persons (99%), history of vaccination in 1382 (2004) in 188 persons (95%), history of measles infection in 14 persons (7%), history of measles in family in 17 persons (8%), history of contact with measles case in 19 persons (9%) and history of fever in time of vaccination in 1 person (0%) was positive. Anti measles IgG antibody in 174 persons (88%) was positive, in 19 persons (9%) was negative and in 3 persons (1.5%) was equivocal.

**Conclusion:** In our study IgG level is lower than to prevent small epidemics. Because medical students are living in high risk area. We suggest that evaluate anti measles IgG antibody then prescribe vaccine for who is IgG negative on equivocal.

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**P1429** Hepatitis B vaccination efficacy among school-age children in south of Iran

M. Moghaddami*, S. Hashemi, K. Bagheri lankarani (Shiraz, IR)

**Objective:** Hepatitis B infection is an important cause of morbidity and mortality in the world wide due to causing cirrhosis and hepatocellular carcinoma. Despite advances in antiviral therapy, only a minority of patients with chronic hepatitis B will have a sustained response. Thus, primary prevention by vaccination to increase herd immunity remains the main thrust in the control of hepatitis B virus (HBV) infection and many countries such as Islamic republic of Iran HBV vaccination has been incorporated into the national expanded program on immunisation.

**Method:** We have assessed the efficacy of vaccine against HBV infection and chronic carriage by examining 392 students (age 6–8 years old) who measured in adults before and one-month after the second dTpa booster immunisation.

**Results:** Ten years following the pertussis booster vaccination, the geometric mean concentrations of IgG elicited by each of the three vaccine antigens had decreased from the five-year post-vaccination levels, but 100% of the adults still had detectable FHA-IgG, 96% had PRN-IgG and 65% had PT-IgG. CMI to FHA, PRN and PT was positive in 96%, 57% and 54% of the subjects, respectively. The geometric mean antibody concentrations were back to the same level as 10 years ago before the first booster. A booster IgG response was found in 97% (FHA), 93% (PRN) and 85% (PT) of the subjects in 21 years of age after the second dTpa. CMI levels to FHA, PRN and PT persisted above the pre-booster levels 10 years before and showed significant increase after the second booster immunisation, being positive in 96%, 80% and 80% of the subjects, respectively.

**Conclusions:** The results of the present study in adult indicate that the interval between acellular pertussis booster immunisations might be extended to 10 years. This study supports the use of Boostrix™ as a decennial booster.

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**P1427** Immunity to pertussis 10 years after acellular booster vaccine in adolescence and response to a second dTpa booster in young adults

Q. He*, O. Van Der Meer, L. Mannermaa, A. Linko-Parcinen, J. Mertsoila (Turku, FI; Rixensart, BE)

**Background:** We conducted a 10-year follow-up study on persistence of pertussis specific antibody and cell-mediated immunity (CMI) following booster immunisation of 11-year-old adolescents with a reduced-antigen-content tri-component acellular pertussis vaccine (Boostrix™). The study subjects were re-immunised with the same vaccine at 21 years of age.

**Objective:** To assess the pertussis vaccination status and define its main causative factors; to compare the patient’s self-declared vaccination status with his actual immunisation cover.

**Methods:** Prospective study assessing the tetanus immunisation status based on reliable evidence (injection certificates or point of care quick serodiagnostic test) and its determinants among patients consulting for wound care at the Emergency Department of a non university Hospital.

**Results:** 1120 patients were included. 50% declared they had their boosters in time, 31% didn’t know their vaccination status, and 16% had some kind of vaccination certificate. As for actual coverage, it appeared that only 61% were immunised and 8% not immunised against tetanus, but for 30.5% it was unspecified (no document, no test). The cover decreased significantly with age. It was better for men.

**Conclusion:** According to current data, our findings confirm that only 61% of the population is immunised against tetanus, with a lack of coverage in particular for women and elderly. Therefore we have to change our practices to increase immunisation coverage and reduce the use of specific human immunoglobulins for tetanus prophylaxis.

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**Objective:** To assess the pertussis vaccination status and define its main causative factors; to compare the patient’s self-declared vaccination status with his actual immunisation cover.
had received the vaccine in infancy and 314 students (age 9–10 years old) who had not received it in Sepidan City, a south Iranian city. Also we determined the titer of Anti HBs Ab in vaccinated students. 

**Results:** Among 394 vaccinated students only 2 (0.5%) were HBV infected (HBe Ab positive) and no one were chronic carrier 294 students (63%) had Anti HBs titer of greater than 10 IU/ml 117 students (30%) had Anti HBs titer between 1 to 10 IU/ml and only 28 children had Anti HBs titer less than 1 IU/ml.

Among 314 unvaccinated students 5 person (1.6%) were HBV infected (positive HBc Ab) and 2 students (0.6%) also was chronic carrier (positive HBs Ag).

**Conclusion:** We found vaccination cannot reduce infection rate among vaccinees but has significant effect among reduction of chronic infection and carrier state. Our findings are as same as other studies and emphasized on vaccine role in control of HBV infection control in endemic area.

### Immunogenicity of hepatitis A and B vaccines among liver transplant candidates


**Objectives:** Immunisation against hepatitis A (HAV) and B (HBV) viruses is strongly recommended for liver transplant candidates. The objective of this prospective study was to evaluate the immunogenicity of HAV and HBV vaccines in patients waiting for a liver transplant.

**Methods:** Between March 2006 and March 2008, 100 liver transplant candidates attending our transplant unit were studied for serological markers for hepatitis viruses and received an update of HAV (2 doses 24 weeks apart of 1440 UI of HAV vaccine) and HBV (3 double doses (40 μg) of HBV vaccine at week 0, 4 and 24) immunisation.

**Results:** Their mean age was 51 years (range, 19–66), M/F ratio was 2.5:97% had cirrhosis, mostly due to alcohol abuse (37%), chronic viral hepatitis (35%) or autoimmune disease (14%); 20% had liver carcinoma complicating the cirrhosis. On a declarative basis, only 18% and 11% of them had received HBV and HAV vaccine, respectively. Prevalence of anti-HAV and anti-HCV antibodies was 88%, and 37% respectively. Prevalence of serologic markers of HBV infection was 52%, with isolated anti-HBs in 21 patients. A total of 53 patients had an indication of vaccination against viral hepatitis: 5 for HAV vaccination alone, 4 for HBV vaccination alone, and 7 for both vaccines. Among the 48 patients with no marker against HBV, 16 patients received a liver transplant before vaccine schedule completion (2 before the 1st injection, 4 before the 2nd and 10 before the 3rd). Seroprotection against HBV occurred in only 42% of the vaccinated with the 3 doubles doses. Seroconversion (anti-HAV antibody >20 mIU/ml) occurred in 100% of patients after 2 doses of HAV vaccine.

**Conclusion:** In liver transplant candidates, immunogenicity of HBV vaccine, even using a double dose regimen, is poor. Furthermore, almost one third of the patients have been transplanted before the vaccination was over. Taking together, our results support the recommendations that patients should be proposed HBV vaccine earlier in the course of the liver diseases.

### Various aspects in nosocomial infections

**P1430 Immunogenicity of hepatitis A and B vaccines among liver transplant candidates**

**P1431 Food-borne Salmonella enteritidis outbreak in a mental health institution**


**Objectives:** In the state institution for the mentally impaired (IMI), an acute outbreak of gastroenteritis occurred in over 100 people during a single day. Over the next three days, 407 residents (out of 580) and 15 staff members (out of 350) suffered from vomiting and diarrhoea. All of them ate a bean salad prepared the day before. A total of 39 residents were hospitalised.

**Methods:** Microbiological examination of stool samples, blood cultures in hospitalised patients, PFGE genotyping.

**Clinical:** follow-up of signs and symptoms: body temperature, vomiting, diarrhoea, secondary infection and outcome.

**Results:** Salmonella enteritidis was confirmed from the stool samples of IMI residents and staff members, as well as from the bean salad. PFGE genotyping showed a 96% match between Salmonella isolated from the stool samples and bean salad. Two months after the outbreak, 50 residents (12.3%) were still Salmonella carriers, but only one was a Salmonella carrier a year later.

In all, 369 residents had an acute enteric infectious gastrointestinal illness with vomiting and diarrhoea, 306 of them also with fever. 38 residents were only febrile without vomiting and diarrhoea but with positive stool samples. They all needed rehydration: 208 residents needed intravenous rehydration and the addition of other substances. A total of 39 residents with serious morbidity and mortality risk were hospitalised, 20 of whom (51.3%) were given antibiotics. There were positive blood cultures in two hospitalised residents. Four residents with severe underlying diseases died (mortality rate 0.8%), one from septic shock and the others due to pneumonia.

**Conclusion:** The salmonellosis outbreak at the IMI was significant because of the more than 70% attack rate, probably due to high antibiotic consumption and sedatives used in the institution. The residents are mentally impaired and were not able to follow infection control guidelines such as hand washing, hand rubbing and contact precautions strictly. Personnel with acute diarrhoeal disease were promptly removed from resident care activities and non-medical personnel were involved in care activities helping the medical staff stop the spread of salmonellosis. It is important to note that only a few secondary transmissions occurred.

**P1432 Increased risk of postpartum infections after caesarean section compared with vaginal birth**


**Objective:** The prevalence of caesarean section (CS) in Denmark has increased (from 13% of births in 1997 to 22% in 2007), and a substantial part of the women has a CS without medical contraindications to vaginal birth (VB). Infection is the most frequent complication after CS, and may occur after hospital discharge. We compared the risk of urinary tract infection (UTI), postoperative wound infection (PWI) and bloodstream infection (BSI) within 30 days after VB, emergency CS and elective CS, respectively.

**Methods:** We conducted a register-based cohort study including all women giving birth in hospitals in the County of Aarhus, Denmark between 2001–2005. We combined data from various hospital registries to identify postpartum infections. We defined UTI as presence of a positive urine culture with ≥100,000 colony forming units per ml and/or treatment by a physician with a UTI-specific antibiotic. PWI was defined as either presence of a positive culture from the wound or an associated abscess, treatment with dicloxacillin, readmission or reoperation of the patient due to wound infection. BSI was identified as presence of a positive blood-culture with a relevant pathogen and concomitant antibiotic treatment.

**Results:** During the 5-year period we included 32,468 women. Of these, 26,288 (81%) women had a VB and 6,180 (19.0%) had undergone CS. The prevalence proportion of postpartum UTI was 1.5% (403/26,288) after VB and 2.8% (176/6,180) after CS, and the prevalence proportion of PWI was 0.80% (20/26,288) after VB and 5.0% (308/6,180) after CS. Only 0.06% (18/32,468) women had BSI. Women having undergone emergency and elective CS did not differ concerning the risk of postpartum UTI. In contrast emergency CS was associated with a fifty per cent higher risk of having postpartum PWI compared with elective CS when adjusted for well-known risk factors (OR 1.52, 95%CI 1.15–2.02). Seventy-nine per cent (258/328) of PWI and 76% (439/579) of UTI were diagnosed post discharge.

**Conclusions:** The risk of acquiring a postpartum infection was substantially increased after caesarean section compared with vaginal...
Assessment of the treatment and outcomes associated with severe and mild-to-moderate *Clostridium difficile* infection

**S.Wieczorkiewicz¹, J.Joury, L.Danziger, D.McMahon (Chicago, Lexington, US)**

**Objective:** The incidence and severity of *Clostridium difficile* infection (CDI) have increased dramatically in recent years as a result of a more virulent and resistant strain. Thus, it is essential for prompt diagnosis and early treatment interventions to occur. The objectives of this study were to determine the treatment approach for CDI at an urban, teaching institution, identify the incidence of severe disease, and to describe treatment outcomes.

**Methods:** This was a single-centre, retrospective study in hospitalised patients (HP) greater than 18-years-old who received metronidazole (MTR) or oral vancomycin (VAN) for the treatment of CDI over a five-year period (2002–2007).

**Results:** 7,280 HP were identified. Of these, 106 were randomly selected for further review (46% male). 236 treatment observations (TO) were evaluated; 91% MTR use versus 9% VAN use. 64% of CDI cases were considered severe (S) and 36% mild-to-moderate (MM). 84% of S CDI cases were treated (tx) with MTR and 25% of MM CDI cases were tx with VAN. Of S CDI cases, 53% tx definitively (DEF) with MTR, 16% tx DEF with VAN, 31% tx empirically (EMP) with M, 0 tx EMP with VAN. Of MM CDI cases, 61% tx DEF with MTR, 7% tx DEF with VAN, 30% tx EMP with MTR, and 2% tx EMP with VAN. Only 31% of the S cases were treated EMP. The percentage of TO that resulted in a lack of symptom resolution, MTR failure, and recurrence is 41 in both S and MM, 33 S and 20 MM, and 40 S and 41 MM respectively. An overall MTR failure rate of 22% was observed. Recurrence rates increased with each recurrent CDAD episode. By the third episode, patients were 100% likely to experience a recurrence. In 203 (86%) TO, the HP were continued on the offending antimicrobial despite the CDI diagnosis.

**Conclusions:** In our institution, high rates of S disease were observed overall. Most patients were treated with MTR regardless of disease severity and no patients in the S CDI group were tx EMP with VAN. In both the S and MM groups, many CDI cases did not result in symptom resolution. A higher incidence of MTR failures was observed in the S group. Similar rates of recurrence were observed in both groups and recurrence rates dramatically increased with each recurrent CDI episode comparable to that reported in the literature. Causative antimicrobials were rarely discontinued. More attention is needed in identifying those HP at high risk and ensuring these HP receive appropriate treatment to improve outcomes.

**C. difficile** isolates from severe cases of CDI and/or persistent outbreaks for genotyping. PCR ribotyping of the toxin positive **C. difficile** isolates was performed according to the protocol of the Anaerobe Reference Unit in Cardiff. When a local outbreak was suspected, also pulsed-field gel electrophoresis (PFGE) was performed.

**Results:** A total of 740 cases of CDI were reported from 12 hospitals; 514 (69.5%) were nosocomial. Of all CDI cases, 125 (16.9%; range by hospital, 1.5−50.0%) were severe: 84 (11.4%; range, 1.5−50.0%) were related to readmission, 32 (4.3%; range, 0−12.5%) death, 7 (0.9%; range, 0−9.5%) intensive care and 2 (0.3%; range, 0−1.6%) colectomy. The overall rate, nosocomial rate and prevalence at admission was 0.71 per 1000 patient-days (range, 0.10−1.92), 0.49 per 1000 patient-days (range, 0.05−1.15) and 0.71 per 1000 admissions (range, 0.12−2.18), respectively. Of the 12 hospitals having reported cases of CDI, 8 had sent isolates for genotyping. The PCR ribotype 027 was detected in CDI cases of the 8 hospitals: in one of them both the proportion of severe cases and nosocomial rate was high and in the others low or moderate.

**Conclusions:** The surveillance provides the first detailed information on CDI epidemiology in Finnish hospitals, showing major differences between hospitals. However, only minority of isolates from severe cases of CDI were sent for genotyping. This may be due to lack of communication between the infection control staff and clinical microbiology laboratory.

**Resource use and costs associated with **Clostridium difficile** diarrhoea in a university hospital**

**N.Aghe, E.Mattila, V.J.Anttila, M.Kanerva³ (Helsinki, FI)**

**Objectives:** *C. difficile* is one of the most common healthcare-associated infections. It is also associated with increased resource use and costs. We wanted to study the incidence and the economic burden caused by *C. difficile* in the Helsinki University Central hospital during Feb 2007-May 2008.

**Methods:** We conducted laboratorio-based prospective surveillance of healthcare-associated *C. difficile* incidence at six acute care wards during a 16 months’ study period, and reviewed the patient records for symptoms, diagnostic tests and treatment. A case was defined as a symptomatic patient with positive stool sample for *C. difficile* between 3 days after admission and 4 weeks after discharge, and symptoms associated with infection. The data of the resource use was gathered from the patient records. Prolongation of hospital stay was analyzed by appropriateness evaluation protocol (AEP) method. Unit costs for bed days, isolation, diagnostic tests and medication were obtained from the literature, hospital administration, laboratory and pharmacy.

All incremental resource use and costs caused by *C. difficile* infection from hospital perspective were calculated for those patients who were in the hospital at the time of symptoms.

**Results:** The monthly incidence of the *C. difficile* infection varied between the study wards throughout the study period from 0.7 to 3.8 cases/1000 patient days. Altogether 72 patients were included in the cost analyses. The average incremental costs associated with *C. difficile* infection was 2200 €/patient, but the cost data was highly skewed. Incremental cost for hospital was 165 000 € during study period (approximately 1700 €/month/ward). *C. difficile* infection prolonged the stay by a mean of 2.7 days. Most of the recourse use came from extra days (85%). Other cost drivers were incremental ICU days (6%), laboratory (4.5%), medication (1.4%) costs and isolation (1.2%).

**Conclusion:** Healthcare-associated *C. difficile* infection caused significant extra costs for the hospital mostly due to prolongation of hospital stay.

First results of hospital-based surveillance of *Clostridium difficile*-associated infections in Finnish acute care hospitals

**O.Lyytikäinen*, T. Möttönen, S. Kotila, S. Ibrahem, A. Virolainen-Julkunen (Helsinki, FI)**

**Objectives:** In January 2008, a new surveillance module for CDI started as a part of the Finnish Hospital Infection program (SIRO) in collaboration with the national reference laboratory in order to estimate the incidence of CDI in Finnish hospitals, to detect severe cases of CDI and outbreaks.

**Methods:** From January through September 2008, prospective laboratory-based surveillance was conducted using the interim case definitions of the European Centre for Disease Prevention and Control (ECDC) for CDI, origin and severe case of CDI. Clinical and microbiological data were recorded on a standardised form. Patient-days and admissions were obtained from the hospital’s information technology department. We calculated the overall and nosocomial incidence rates of CDI, and prevalence of CDI among admitted patients. All clinical microbiology laboratories in participating hospitals were asked to send
Epidemiology of healthcare-associated Stenotrophomonas maltophilia infections in CF and in ICU patients: role of biofilm formation

R. Cipresso, M. Barchitta, R. Marzagalli, G. Di Bonaventura*, A. Pompilio, G. Gherardi, A. Agodi (Catania, Chieti, Rome, IT)

Objectives: Stenotrophomonas maltophilia is an emerging bacterial pathogen which is currently isolated with increasing frequency from the airways of cystic fibrosis (CF) patients as well as from different sites of intensive care units (ICU) patients. In a previous study S. maltophilia strains isolated from the airways of independent CF patients, have been characterised for the expression of several virulence-associated factors. The present study was designed in order to: i) investigate the clonality, the mode of transmission and the patients’ risk profile for acquisition of S. maltophilia in CF and ICU patients, and ii) to evaluate the epidemiological significance of biofilm formation both in CF-associated and in ICU-associated strains.

Methods: Patterns of S. maltophilia acquisition in the ICU during the period of the survey were carriage, colonisation and infection (ventilator-associated pneumonia, urinary tract infection, bloodstream infection, catheter related infection), characterised using well established definitions. Clonality assessment was performed by PFGE of genomic DNA digested with 25 U/ml of XbaI. Macrorestriction fragments were separated using a CHEF-DR III apparatus and genomic relatedness performed using Tenover criteria. Cross-transmission was assumed when two patients had indistinguishable isolates. Biofilm formation was assessed by crystal violet staining in poly styrene 96-well microtiter plates after 24 h of incubation at 37°C.

Results: A total of 42 CF-associated isolates and of 38 ICU-associated isolates were subjected to macrorestriction analysis. A total of 32 different PFGE profiles were observed among CF isolates. Twelve distinct clones were identified among the ICU isolates, six associated with cross-transmission of infection and/or colonisation; a major clone (named clone A) was responsible for the epidemiic spread of S. maltophilia. Notably, the degree of biofilm formation was shown to be significantly higher in ICU strains than in CF strains (p < 0.05).

Conclusion: Health care-related infections are associated with high attributable mortality. S. maltophilia is an important alert organism increasingly isolated in both CF and in ICU patients: increasing levels of antibiotic resistance and the ability to form biofilms surrounding invasive devices pose special challenges to be addressed for appropriate control strategies.

Creating a collaborative network for the study of bacteremia in Denmark: frequency of recurrence with the same and different micro-organisms

U.S. Jensen*, C. Østergaard, H.C. Schunheyder, J.D. Knudsen (Copenhagen, Aalborg, DK)

Objectives: In most countries bacteremia is a reportable infection only if the causative agent is subject to national surveillance. In a few countries all cases of bacteremia are reportable but only one episode per microorganism is included per year. Therefore population-based data are sparse especially with regard to the epidemiology of recurrent bacteremia. We present data from a newly established collaborative network in Denmark with prospective registration of bacteremia in a population of approximately 1.7 mill. inhabitants.

Methods: Three departments of clinical microbiology (DCMs) participate in the network (Hvidovre Hospital and Herlev Hospital, The Capital Region and Aalborg Hospital, North Denmark Region). Bacteremia is defined as a clinical episode with one or more positive blood cultures (BCs) given significance by a clinical microbiologist and the attending physicians. A recurrence was defined as a positive BC with the same microorganism/s as obtained ≥30 days or with another microorganism/s ≥48 hours after the first positive BC, respectively. We included all patients with bacteremia during 2006–2007 and follow-up extended for 6 months or until death.

<table>
<thead>
<tr>
<th>Microorganism of the first bacteremia</th>
<th>Number of first episode, 2006–2007</th>
<th>Recurrence, same microorganism (%)</th>
<th>Any recurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>358 247 254 14 (5.9) 7 (2.8) 3 (1.2)</td>
<td>59 10 (1.6) 5 (2.0) 1 (0.7)</td>
<td>84 17 (2.0) 2 (0.7)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>171 134 61 0 (0) 1 (1.0) 0 (0)</td>
<td>15 0 (0) 1 (1.0)</td>
<td>21 0 (0) 1 (0.5)</td>
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<td>Enterococcus faecalis</td>
<td>140 90 53 8 (5.7) 1 (0.7) 0 (0)</td>
<td>8 (5.6) 0 (0)</td>
<td>16 (11.4) 0 (0)</td>
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<td>Pseudomonas aeruginosa</td>
<td>79 63 63 1 (1.2) 0 (0) 1 (1.6)</td>
<td>5 (8.0) 0 (0) 3 (5.0)</td>
<td>13 (2.0)</td>
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<tr>
<td>Candida spp</td>
<td>59 56 58 0 (0) 1 (1.0) 0 (0)</td>
<td>5 (3.4) 0 (0)</td>
<td>12 (6.9) 0 (0)</td>
</tr>
<tr>
<td>Non-haemolytic streptococcus</td>
<td>134 134 61 0 (0) 1 (0.7) 0 (0)</td>
<td>13 (9.7) 0 (0)</td>
<td>17 (13.2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>173 191 200</strong> 2 (1.0) 2 (1.0) 2 (1.0)</td>
<td><strong>173 191 200</strong> 2 (1.0) 2 (1.0) 2 (1.0)</td>
<td><strong>173 191 200</strong> 2 (1.0) 2 (1.0) 2 (1.0)</td>
</tr>
</tbody>
</table>

Conclusion: The frequency of recurrent S. aureus and S. pneumoniae bacteremia was lower than reported in previous studies. We found only small differences among major pathogens in the frequency of recurrence with the same microorganism. However, recurrent bacteremia with any microorganism is common subsequent to K. pneumoniae and E. faecalis bacteremia and to a lesser extent candidaemia. This suggests that host factors and therapeutic regimens in these patients should be targeted in further studies in order to optimise bacteremia management.

Predictors of mortality in patients with ventilator-associated pneumonia: a meta-analysis

I. Siempkas *, K. Vardakas, C. Kyriakopoulos, T. Ntaidou, M. Falagas (Athens, GR)

Objective: Studies exploring predictors of mortality in patients with ventilator-associated pneumonia (VAP) produced conflicting results.

Methods: Potentially eligible reports were searched in PubMed, EMBASE, CINAHL and HEALTHSTAR with no language restrictions. Eligible studies were studies that enrolled only patients with microbiologically confirmed VAP and reported on mortality.

Results: Twenty-one reports were included. Factors associated with mortality were malignancy (OR = 2.85; 95% CI 1.19 to 6.82) at intensive care unit admission as well as inappropriate initial treatment (i.e. treatment either in vitro inactive against the causative bacteria or administered later than 24 hours after diagnosis of VAP) (OR = 2.77; 95% CI 1.95 to 3.94), shock (OR = 4.98; 95% CI 2.65 to 9.38), sepsis (OR = 4.77; 95% CI 2.22 to 10.25), disease severity and sepsis-related organ failure score at the day of diagnosis of VAP. Isolation of non-fermenting Gram-negative bacteria in general (OR = 1.71; 95% CI 1.09 to 2.68) and Acinetobacter baumannii in specific (OR = 1.67; 95% CI 1.02 to 2.73) was also associated with higher fatality; whereas, isolation of Streptococcus pneumoniae was linked to lower mortality.

Conclusion: These findings may explain the variability in mortality across studies on VAP, may help investigators to formulate relevant predicting scores, and may further motivate clinicians to provide appropriate initial treatment.
Epidemiology of candidaemia and antifungal susceptibility patterns in an Italian medical-surgical intensive care unit between July 2003 and June 2008

A.M. Azzini*, F. Boccafoglio, G. Lo Cascio, E. Concia (Verona, IT)

Objective: To evaluate epidemiological trends of candidaemia (CA) and to identify risk factors for it in critically ill patients; to define antifungal susceptibility patterns inside our intensive care unit (ICU) in order to optimise both chemotherapy and prevention of drug resistance of this infection.

Methods: We retrospectively identified all cases of CA and collected their demographic-clinical/laboratory data, in particular the presence of medical devices and antibiotics/antimycotics administration within the 3 weeks before a CA episode. We also considered if CA was CVC-related. Antifungal susceptibility patterns were collected and empirical treatment’s adequacy was evaluated as well.

Results: 58 CA were identified but only 51 were evaluable (all nosocomial, 89% ICU-acquired) The overall incidence of CA was 29.5/10000 patient-days, with a peak during 2004 (37.9/10000 patient-days) and 2005 (32.8/10000 patient-days). C. parapsilosis was isolated in 47% of CA, followed by C. albicans (29%), C. glabrata (12%), C. tropicalis (7%), C. guilliermondii (3%) and C. sake (2%). Non-albicans species remained the more frequent pathogens during 2004–2005–2006, only in 2007 we observed a reversal of trend. 100% of C. albicans and C. tropicalis were fluconazole-sensible (S), on the contrary C. parapsilosis resulted fluconazole-R in only 33% of cases, in 60% SDD and in 7% resistant (R). Most of R or SDD C. parapsilosis were isolated 3 weeks before its onset. The antifungal susceptibility patterns were collected as well.

Results: 100 episodes of CA were identified. The average incidence was 0.99/10000 patient-days/year, ranging from 1.2 in 2005 to 1.1 in 2007. 54% of CA occurred in ICUs, followed by surgical (30%) and medical (16%) wards. Most common predisposing factors were antibiotics (96%), CVC (94%), bladder catheter (93%), total parenteral nutrition (64%), mechanical ventilation (62%) and surgery (58%); steroids accounted for only 32% of cases, no-one was neutropenic or transplant recipient. C. albicans was isolated in 53% of cases, followed by C. parapsilosis (21%), C. glabrata (9%) and C. tropicalis (9%); non-albicans species never exceeded C. albicans (Table 1).

Conclusions: We described an epidemic outbreak by C. parapsilosis in 2004–05, as demonstrated by DNA-fingerprinting. This epidemic was faced by the implementation of more strict infection control measures (hand-hygiene and CVC-management guide-lines). Because of the frequency of fluconazole-R or SDD C. parapsilosis we recommended to start CA treatment with an amphotericin B formulation.

Epidemiology of candidaemia in non-neutropenic patients in an Italian tertiary-care hospital between 2005 and 2008

A.M. Azzini*, C. Recchia, A. Tedesco, E. Concia (Verona, IT)

Objective: To evaluate epidemiological trends in candidaemia (CA) between 2005 and 2008.

Methods: All cases of CA were identified retrospectively; demographic and clinical data were collected, together with data about the use of antifungal/antibacterial drugs, and others predisposing factors, within the 3 weeks before its onset. The antifungal susceptibility patterns were collected as well.

Results: 100 episodes of CA were identified. The average incidence was 0.99/10000 patient-days/year, ranging from 1.2 in 2005 to 1.1 in 2007. 54% of CA occurred in ICUs, followed by surgical (30%) and medical (16%) wards. Most common predisposing factors were antibiotics (96%), CVC (94%), bladder catheter (93%), total parenteral nutrition (64%), mechanical ventilation (62%) and surgery (58%); steroids accounted for only 32% of cases, no-one was neutropenic or transplant recipient. C. albicans was isolated in 53% of cases, followed by C. parapsilosis (21%), C. glabrata (9%) and C. tropicalis (9%); non-albicans species never exceeded C. albicans (Table 1).

Conclusions: The overall annual incidence of CA was high, but stable during the study period. Compared with a similar study conducted between 1992–2001, it showed an increased number of CA inside medical wards (16% vs 8%), and a significant reduction inside ICU (54% vs 65%). Interestingly inside surgical wards remained quite constant (30% vs 27%), in spite of the increasing number of surgical patients. CVC related CA decreased (30% vs 43%) instead of the widest use of CVC. If only 12% of patients with CA received an antifungal prophylaxis, the quite constant number of cases could be attributable to a sort of balance between the increasing patients’ critical-status and the improved infection control policy, particularly inside ICU.

Although the consumption of fluconazole was high, C. albicans remained the predominant species (save for the first half of 2008), but we observed an increasing number of C. parapsilosis resistant to fluconazole.
Infections and mortality of obese patients in the intensive care unit
G. Kallisti, M. Komposa*, M. Michaila, M. Charitidi, P.M. Clouva-Molyvdas (Athens, GR)

Obesity is a well-recognized risk factor of morbidity and cardiovascular mortality. Data concerning critically ill patients are often conflicting. Objective: The aim of this study was to investigate the impact of obesity on the occurrence of infections and mortality in the intensive care unit (ICU).

Patients-methods: All patients admitted to our general ICU were prospectively enrolled in the study. Information recorded included: demographics, APACHE II score at admission in the ICU, body mass index (BMI), number of infectious episodes during ICU LOS, ICU LOS and ICU outcome. Patients with BMI < 20, 20–24.9, 25–29.9 and ≥30 kg/m² were classified as underweight, normal weight, overweight and obese, respectively. Data analysis was performed with logistic regression and a statistical significance level at p < 0.05.

Results: One hundred two patients (64 males, 38 females) were included in the study. Age (mean ± SD) was 51.1 ± 18.5 years, BMI 29.0 ± 5.8 kg/m², APACHE II score 19.5 ± 6.7. There were 3 underweight, 17 normal weight, 46 overweight and 36 obese patients. Underweight and normal weight patients were merged into a single category (non-obese patients). One hundred forty infectious episodes were recorded in 63 patients. Ventilator-associated pneumonia (VAP) and bloodstream infections (BSI) were the most common types of infection (40% and 45% of infectious episodes, respectively). There was no significant difference in VAP occurrence rate or in time to VAP resolution according to BMI category. Obese patients developed marginally more BSI episodes compared to non-obese (2.2 ± 0.8 vs. 1.2 ± 0.4 BSI episodes, respectively, p = 0.043). Crude mortality rate was 35.3% in non-obese, 31.1% in overweight and 10.0% in obese patients (p for linear trend = 0.033). In a logistic model adjusted for age, gender and APACHE II score at admission in the ICU, obese patients had 82% lower probability of ICU death compared with non-obese ones (p = 0.047).

Conclusions: In our patient sample, obese patients displayed an increased occurrence rate of BSI and a lower mortality rate compared with non-obese patients.

Molecular typing is a cornerstone for infection control in neonatology: a case with extended-spectrum β-lactamase Escherichia coli and Staphylococcus aureus
S. Melin*, M. Toepfer, S. Wetterbrandt, G. Rensfeldt, S. Lofgren (Jonköping, SE)

Objectives: Neonatal wards are extreme environments from the infection control perspective. When an outbreak of extended-spectrum β-lactamase (ESBL)-producing E. coli was suspected in our neonatology unit, isolates were characterised and follow-up cultures were obtained. Simultaneous recovery of S. aureus of a previously recognized spa type in other samples further catalysed our analysis. Were these infections sporadic or was there a general breach in hygiene practice?

Methods: Weekly and discharge rectal screening was performed on all in-patients after two cases of infection with ESBL-producing E. coli were identified in June 2008. Thereafter, ESBL-positive children were screened monthly. Parents and staff at the neonatal ward were screened and environmental samples were collected. Concurrent identification of S. aureus infections in two neonates prompted epidemiological characterisation. Pulsed-field gel electrophoresis (PFGE) and spa typing were used to discriminate isolates of ESBL-producing E. coli and S. aureus, respectively.

Results: A total of 118 neonates (430 samples during 6 months) were screened for ESBL-producing E. coli. Fourteen individuals had ESBL-producing E. coli: two infected neonates, six intestinally colonised neonates, two infants of two colonised children, and two staff. One E. coli type caused infection in two children and colonised the intestine of three children and one staff member. At least four of the children had positive cultures at three months follow-up. Two S. aureus isolates from infected neonates were of spa type t091. No S. aureus were found in the environment at this time. Six months earlier, isolates of this spa type infected two neonates and were found in six of seven S. aureus positive environmental samples.

Conclusion: Several different ESBL-producing E. coli types were recovered in the neonatal ward, one of which spread nosocomially. Simultaneously, there was a S. aureus type spreading, indicative of a nosocomial transmission. Molecular characterisation helped to quickly identify the scope of the problem with these common pathogens in this unit and became the baseline for further infection control efforts.

Bone biopsy in guiding appropriate antimicrobial usage in chronic osteomyelitis and improving microbiological surveillance of chronic bone infection in orthopaedic surgery
R. Ferretto*, A. Carlotto, F. Marranconi (Schio, IT)

Objective: Appropriate antimicrobial usage is a key issue of any antibiotic policy. Antibiotics susceptibility testing of the causative organism(s) are mandatory in treating chronic osteomyelitis for required protracted therapy with valuable antimicrobials for hospital environment and risk of antibiotics misuse. The aim of the present observational study was to enforce appropriateness of antibiotic therapy selecting the best specimen for chronic osteomyelitis bacterial diagnosis.

Methods: From January 2004 to October 2008, retrospective review of cultures results of intraoperative bone biopsy from 21 patients admitted to our 462-bed general hospital, were compared with cultures from swabs of fistulae and surgical wounds taken from the same patients, 4 weeks before intraoperative bone biopsy for surgical debridement or devices removal. Discontinuance of any antibiotic therapy 48 hours before bone biopsy was required. Patients with bone infection secondary to diabetic foot, decubitus ulcers or open fractures were excluded.

Results: Bacterial isolates obtained from overall specimens were 39. We found only 33% bacterial concordance between swab and intraoperative bone biopsy specimens. Antibiotic therapy guided by swab specimens antibiogram was inappropriate in 67% of patients. After cultures results from bone biopsy antibiotic streamlining was done in 14 of 21 patients. We found from bone biopsy 5 cases of polymicrobial infection (23%): one included B. fragilis; another patient presented polymicrobial infection including Enterobacter spp. and E. faecium with Van B phenotype (teicoplanin susceptible). Gram-negative rods were isolated in 22% of patients (all from bone biopsy); bone biopsy culture from 1 patient yielded to growth of P. aeruginosa resistant to carbapenems and ciprofloxacin whereas superficial swabs cultures were misleading showing monomicrobial growth of MRSA. From bone biopsy MRSA were isolated in 6 cases whereas from 4 of same patients superficial swabs cultures yielded to growth of MSSA.

Conclusions: Precise identification of pathogens using correct specimen for bacterial diagnosis, is a cornerstone of antibiotic therapy. Prescribing ineffective antibiotic or untreated bone infection due to unrecognized P. aeruginosa carbapenems resistant or delayed identification of Van B phenotype E. faecium neglecting isolation precautions, have serious epidemiological and clinical consequences regarding spreading antibacterial resistance and hospital costs.

Evaluation of device-associated nosocomial infection rates in intensive care units of a university hospital in Turkey
N. Erben*, S. Nayman Alpat, E. Doyak Kartal, I. Osgunes, G. Usluer (Eskişehir, TR)

Objective: To describe the incidence of device-associated nosocomial infections in intensive care units (ICU) of a university hospital in Turkey.

Methods: We performed a prospective nosocomial infection surveillance in nine ICUs of a university hospital between January and December 2008. Nosocomial infections were identified using the Centers for Disease Control and Prevention National Nosocomial Infections Surveillance (NNIS) system definitions. Device-associated nosocomial infection rates were calculated.

Various aspects in nosocomial infections
Results: During the study, we collected data from 5937 patients hospitalised in ICUs for a total 26577 patient days, 7622 central vascular catheter days, 4678 mechanical ventilator days, and 18083 urinary catheter days. The overall nosocomial infection rates were 8.08% (480/5937) and 1.81 per 1000 patient days (480/26577). Central venous catheter-associated blood stream infection (CA-BSI) rate was 14.69 per 1000 device days (78/5314) and was correlated by the CDC National Nosocomial Infections Surveillance system definitions. A total of 285 infections were identified in 187 patients (35.9%). The most commonly observed nosocomial infections were hospital acquired pneumoniae (HAP) (35.3%) (including ventilator associated pneumoniae (VAP), 14.7%), urinary tract infections (UTI) (23.1%), blood stream infections (BSI) (15.7%), surgical site infections (15.7%) and the others (10.8%). Frequently isolated microorganisms were Staphylococcus aureus (22.1%), Actinobacter spp. (18.5%), Candida spp. (14%) and E. coli (11%). Median length of stay of patients with NIs and those without NIs in ICUs were 15.17±10.24 and 7.56±4.89 days, respectively. Extra length of stay of patients with NIs was found 7.6 days. In our study there was a significant relation between NIs and mortality rates (P < 0.05). Mortality rates concerning the infection sites were 66/95 (69.5%) for HAPs (VAPs, 37/42 (88.1%), 28/45 (62.3%) for BSIs and 31/66 (49.2%) for UTIs. The result of univariate analysis showed that NIs were an independent risk factor. Attributable total cost for patients with NIs was 5460 Euros, whereas it was 2452 Euros for patients without NIs. Thus, a patient’s acquiring a NI in ICUs costs an extra 3008 Euros.

Conclusions: Although we found that device utilisation rates in our ICUs were lower than those reported by the NNIS system and International Nosocomial Infection Control Consortium (INICC), VAP and CA-BSI rates were similar to those reported by the INICC while higher than NNIS rates. CA-UTI rates were within acceptable limits, considering the NNIS and INICC rates.

Table I. Device use and incidence densities for specific device-associated infections according to the types of ICUS

<table>
<thead>
<tr>
<th>Type of ICU</th>
<th>No. of patients</th>
<th>Patient-days</th>
<th>Device utilisation</th>
<th>Rate per 1000 device days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaesthesia</td>
<td>465</td>
<td>2689</td>
<td>0.41</td>
<td>0.47 0.67 29.73 31.45 15.57</td>
</tr>
<tr>
<td>Coronary</td>
<td>1161</td>
<td>3430</td>
<td>0.08</td>
<td>0.05 0.40 30.53 0 7.25</td>
</tr>
<tr>
<td>Medical</td>
<td>756</td>
<td>3141</td>
<td>0.08</td>
<td>0.18 0.69 0 16.33 12.87</td>
</tr>
<tr>
<td>Surgical</td>
<td>1554</td>
<td>6194</td>
<td>0.07</td>
<td>0.44 0.79 28.37 11.78 5.11</td>
</tr>
<tr>
<td>Cardiothoracic</td>
<td>692</td>
<td>1809</td>
<td>0.09</td>
<td>0.26 0.65 41.1 1.7 7.51</td>
</tr>
<tr>
<td>Respiratory</td>
<td>239</td>
<td>1866</td>
<td>0.38</td>
<td>0.13 0.95 37.97 8.03 3.40</td>
</tr>
<tr>
<td>Neurology</td>
<td>187</td>
<td>1156</td>
<td>0.25</td>
<td>0.21 0.98 17.12 8.33 15.90</td>
</tr>
<tr>
<td>Neurosurgical</td>
<td>450</td>
<td>3128</td>
<td>0.22</td>
<td>0.39 0.91 37.15 11.45 3.88</td>
</tr>
<tr>
<td>Burn</td>
<td>71</td>
<td>1089</td>
<td>0</td>
<td>0.02 0.26 0 0 3.6</td>
</tr>
<tr>
<td>Paediatric</td>
<td>362</td>
<td>2083</td>
<td>0.38</td>
<td>0.31 0.31 16.52 13.78 13.82</td>
</tr>
<tr>
<td>Total</td>
<td>5937</td>
<td>26577</td>
<td>0.29</td>
<td>0.28 0.68 27.79 14.69 7.63</td>
</tr>
</tbody>
</table>

ICU, intensive care unit; MV, mechanical ventilator; CVC, central vascular catheter; UC, urinary catheter; VAP, ventilator-associated pneumonia; CA-BSI, central venous catheter-associated blood stream infection; CA-UTI, catheter-associated urinary tract infection.

Background and Objectives: Osteomyelitis is a progressive infection of bone, that results in inflammatory destruction of the bone, bone necrosis, and new bone formation and may progress to a chronic and persistent state. Orthopedic injuries suffered by casualties during combat constitute approximately 65% of the total percentage of injuries and are evenly distributed between upper and lower extremities. The aim of this study was management of infections.

This study carried out by Doctors Without Borders (MSF group) in Imam Hosein Hospital of Mehran. This city located in Ilam province, near the border of Iran and Iraq. During our study from April 2008 to November 2008 Sixty three Iraqi patients with compact related osteomyelitis hospitalised in Imam Hosein hospital. Management strategies of combat-related injuries primarily focus on early Surgical debridement and stabilisation, antibiotic administration, and delayed primary closure. Herein, we provide evidence-based recommendations from military and civilian data to the management of combat-related injuries of the extremity. Specimens collected and sent to Milad Hospital of Tehran for culture and susceptibility testing. Isolated microorganisms identified by conventional bacteriological methods and susceptibility testing performed by disk diffusion methods as recommended Clinical Laboratory Standards Institute (CLSI).

Conclusions: From April 2008 to November 2008 Ninety-six specimens obtained from patients hospitalised in Imam Hosein hospital of Mehran city in border of Iran and Iraq. All specimens were sent to Milad Hospital of Tehran for culture and susceptibility testing. Isolated microorganisms identified by conventional bacteriological methods and susceptibility testing performed by disk diffusion methods as recommended Clinical Laboratory Standards Institute (CLSI).
**P1447** Long-term trends in the occurrence of adult blood stream infection

X. Ma*, Y. Li, M. Zhang (Beijing, CN)

**Objective:** Blood stream infections (BSI) are a major cause of morbidity and mortality in developing countries. Reports have suggested that the epidemiological profile of invasive bacteria infections is changing. We sought to determine trends in the occurrence of adult blood stream infection at Peking University first Hospital in north China.

**Methods:** The medical records of all cases of BSI were manually reviewed by the experienced infectious diseases investigator to confirm the diagnosis. Data were collected including demographic features, underlying diseases, presence of invasive devices (central venous catheters, urinary catheters, endotracheal tubes), and all clinical and laboratory data pertaining to infection during 2000–2007.

**Results:** There were 231 cases; the mean±SD age was 59±21.3 years, and 59% were male. Male bacteraemia was the most common source (48.4%), followed by respiratory tract (19.9%), urinary tract (12.6%), and skin sources (6.0%). The most common organisms identified were Escherichia coli (in 89 patients with BSIs [38.5%]), Staphylococcus aureus (in 36 patients with BSIs [15.6%]) and Klebsiella pneumoniae (in 25 patients with BSIs [10.8%]). Fungi, mainly Candida species, accounted for 9.1%, and Streptococcus, spp. for 4.8%. Significant increases occurred between 2000 and 2007 in community-acquired BSI (from 1.6 to 8.3/10000 admissions, P <0.01) and nosocomial BSI (from 6.3 to 10.7/10000 admissions, P=0.01). The most frequent concomitant medical conditions were diabetes mellitus (30.2%) in community-acquired BSI patients.

**Conclusions:** The community-acquired blood stream infection rate in the hospital has sharply risen in the past 7 years, possibly partly due to increased diabetes mellitus.

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**P1448** Characteristics of infections associated with external drainage devices of cerebrospinal fluid: a retrospective analysis over 12 years

L. Walti*, J. Coward, A. Conen, A. Trampuz (Basel, CH)

**Objectives:** External drainages devices (EDD) are used to treat transitory hydrocephalus. EDD infections often resemble the underlying neurological condition, mainly intracranial bleeding, and data on EDD infections are limited. We therefore retrospectively analyzed EDD infections in adults at our institution.

**Methods:** Hospitalised patients aged ≥18 y presenting with an EDD infection from 01/98 through 12/08 were included. EDD infection was defined as methicillin-resistant *Staphylococcus aureus* in 36 patients with BSIs [15.6%]) and Klebsiella pneumoniae (in 25 patients with BSIs [10.8%]). Fungi, mainly Candida species, accounted for 9.1%, and Streptococcus, spp. for 4.8%. Significant increases occurred between 2000 and 2007 in community-acquired BSI (from 1.6 to 8.3/10000 admissions, P <0.01) and nosocomial BSI (from 6.3 to 10.7/10000 admissions, P=0.01). The most frequent concomitant medical conditions were diabetes mellitus (30.2%) in community-acquired BSI patients.

**Conclusions:** The community-acquired blood stream infection rate in the hospital has sharply risen in the past 7 years, possibly partly due to increased diabetes mellitus.

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**P1449** Comparison between multi-locus variable number of tandem repeats and pulse field gel electrophoresis for typing of nosocomial *Pseudomonas aeruginosa* isolates

R. Vatcheva-Dobrevski*, J. Ivanov, E. Dobrexa, T. Kantardjiev (Sofia, BG)

**Objectives:** To compare the performance (discriminatory power, typeability and reproducibility) of traditional PFGE and MLVA6 for molecular typing of hospital *P. aeruginosa* isolates.

**Methods:** A total of 81 non-repeat clinical *P. aeruginosa* isolates were included in the study collected at two university and two regional hospitals during 2004–2008. All isolates were identified as conventional as well as automated (API or VITEK II, BioMerieux, France.) identification systems. PFGE molecular typing was performed with SpeI according to a standardised protocol and the E. coli O157:H7 was added in the electrophoresis buffer for preventing DNA degradation. Six previously described VNTR loci were combined in the MLVA assay and the fragment analysis was performed on a capillary electrophoresis system. Data and cluster analysis was carried out in Bionumerics v4.5 (Applied Maths, Belgium)

**Results:** Both methods were 100% reproducible whereas typeability was 99% and 97.5% for PFGE and MLVA, respectively. The clustering of the strains with the two methods was surprisingly similar with approximately 85% concordance. A total of 21 (D=0.95) types were distinguished by the MLVA assay versus 18 (D=0.93) by PFGE. The genotype distribution appeared to be non-hospital dependent. As expected, most of the genotypes were associated with different wards at the two university hospitals but several types were located in all hospitals. In general the two methods performed similarly although MLVA was more discriminating.

**Conclusions:** Although PFGE is technically demanding and costly it is still the referent and most often applied method for typing of nosocomial isolates. With this study we demonstrate that a simplified MLVA alternative could be as equally effective as PFGE but far more rapid and cost-effective for typing *P. aeruginosa*.

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**P1450** Risk factors for hospital infections with multidrug-resistant bacteria in patients with cancer

E. Sahin, G. Ersoz*, M. Goksu, S. Karacorlu, A. Kaya (Mersin, TR)

**Objectives:** Infections with multidrug-resistant (MDR) bacteria have been linked to increases in morbidity, length of hospitalisation, healthcare cost, and mortality. We aimed to determine the prevalence, risk factors, and outcomes of nosocomial infection due to MDR bacteria in patients with cancer.

**Methods:** Design: Retrospective observational cohort study of prospectively collected data. All patients who had nosocomial infection in patients with cancer between January 2004 to December 2008 at the University of Mersin through searching the microbiology and the Infection Control Surveillance records were identified retrospectively. All clinical specimens were performed following standard procedures. Biological isolates were identified with the help of API (bioMérieux sa Marcy l’Etoile, France). MDR bacteria were defined as mexitellicin-resistant *Staphylococcus aureus* (MRSA), ceftazidime- or imipenem-resistant *P. aeruginosa*, A. baumannii, Stenotrophomonas maltophilia, and extending spectrum β-lactamase producing Gram negative bacilli.
SPPS 11.5 (Chicago, IL) package was used for statistical analysis. Univariate statistical analysis and Multivariate logistic regression analysis were performed to determine risk factors independently associated with the development of MDR bacteria infections. P values of <0.05 were considered to indicate statistical significant.

Results: A total of 219 patients with cancer were analyzed; 61% were male, 145 (66.2%) were solid malignancies, 74 (33.8%) haematologic malignancies, 57 (26%) were in ICU wards and the mean age was 50.51±21.13 years. Of these patients, 82 (37.4%) had nosocomial infections due to MDR bacteria. Overall mortality was higher in patients with MDR bacteria infections (37.8%) than patients with non-MDR infections (21.1%) (p=0.008).

Multivariable logistic regression analysis revealed that duration of hospital stay (p=0.001, OR=1.045, CI%95 1.018–1.072), solid malignancy (p=0.029, OR=3.961, CI%95 1.151–13.631), mechanical ventilation (p=0.008, OR=5.404, CI%95 2.855–10.591), tracheostomy (p=0.044, OR=6.276, CI%95 1.055–37.333), and broad spectrum antibiotic use (p=0.000, OR=4.089, CI%95 1.912–8.744) were independently associated risk factors for infections with MDR bacteria.

Conclusion: Among cancer patients, hospital infection caused by MDR bacteria occurred more frequently in patients with solid malignancy, invasive procedures, receiving broad spectrum antibiotic and prolonged length of hospital stay.

**P1451** Dimension of nosocomial infections in a burn care centre: analysis of 7-year active surveillance

O. Oncul, E. Ulbar, A. Acar*, F. Yuksel, S. Cacusla, L. Gorenek

(Istanbul, TR)

Objectives: To determine the trends of nosocomial infection (NI), aetiological agents and risk factors in burned patients.

Methods: Data were collected prospectively from 456 burned patients admitted to a Turkish Burn Care Unit (BCU) between 2001 to 2008. A researched assistant reviewed the medical record for each patients by active surveillance according to CDC and NNIS criteria.

Results: Among 456 burned patients, 329 cases acquired 436 NI (11.0% pneumonia, 52.0% burn wound infection (BWI), 5.5% urinary tract infection and 31.5% bloodstream infection) for an overall NI rate of 25.8 per 1,000 patient-days. The mean hospitalisation time (46.2±14) and the mean body surface area burned (BSAB) (37.4±11) of the patients with NI were higher than those with non-NI (22.3±9.3), (25.8±9.1) and (16.4±4.7) (P=0.001, P=0.01, P=0.001) respectively. Of all the patients, 81 (17.8%) died. Of all the patients, 81 (17.8%) died. A. baumannii (35.9%) and P. aeruginosa (11.4%) were most common bacteria identified in 396 strains. The most effective antibacterial agents for A. baumannii was meropenem (75%) and, that for P. aeruginosa was meropenem (75%) and P. aeruginosa (44.7%), A. baumannii (35.9%) and Staphylococcus aureus (11.4%) were most common bacteria identified in 396 strains. The most effective antibacterial agents for P. aeruginosa were meropenem (75%) and, that for A. baumannii was neutromycin (93%) respectively. Thirteen (7.3%) strains of P. aeruginosa and 16 (11.3%) strains of A. baumannii were panresistant to all antibiotics. In S. aureus strains, 38.6% were methicillin resistant.

Conclusions: Considering the high incidence of BWI, high mortality rate and panresistant strains in our BCU, more strictly infection control policies and comprehensive education campaining are required.

**P1452** Use of drotrecogin alpha in a surgical septic cohort of patients


(Alicante, ES)

Objectives: Drotrecogin alpha is a drug used in high risk patients with severe sepsis and septic shock, defined by an Acute Physiology and Chronic Health Evaluation (APACHE II) score in the United States indication more than 25 and at least 2 acute organ dysfunctions in the European Union indication (SOFA). Drotrecogin alpha is considered to have a broad range of effects, not only on multiple stages of the coagulation cascade, but also as a fibrinolitic agent and as an anti-inflammatory agent. The main side effect is the risk of bleeding due to the inactivation of the V and VIII coagulation factors. In both clinical trials and “real practice” registries the proportion of surgical patients is always small. After surgery there is a 12 hour window for the initiation of the therapy with drotrecogin alpha, even though the risk of bleeding continues during all the treatment.

Methods: We analyzed retrospectively all the patients (78) admitted to the Surgical Critical Care Unit with surgery in the previous 30 days and that were treated with drotrecogin alpha between June 2003 and November 2008.

Results: We included 78 patients. The age was 62.4±16.9 (69.2%) male. The severity score measured by the APACHE II was 20.2±5.0 and the SOFA was 3.3±1.0 (98.7% had 2 or more organ dysfunction). The delay from the diagnosis of the sepsis until the initiation of the therapy was 22.4±16.5 hours. The origin of the patient was in the 51.3% nosocomial, and the surgery was emergency in the 88.5% of the cases. The type of surgery was in the 74.4% abdominal, 5.1% cardiac, 6.4% vascular, 3.8% other. The infection site was in the 46.2 peritonitis, 29.5% pneumonia, 3.8%, mediastinitis and 20.8 other. During the sepsis episode other coagulants used were: Insulin perfusion (60.3%), corticoids (38.5%) and prophylactic low weight heparin (78.2%). The 88% of the patients didn’t received renal replacement. The 28 day mortality was 42.3%. We found 4/78 serious bleeding complications (5%), two of them fatal.

Conclusion: We need specific trials in the subgroup of surgical patients, especially nosocomial ones, due to the high mortality. The use of different therapies to lower the mortality is encouraged. In our cohort the survival rate of patients treated with drotrecogin alpha was 57.7% with a low incidence of bleeding if we apply the measures related to the selection of patients, control of abdominal drains and coagulation times.

**P1453** External ventricular drain infections

J.D. Ruiz-Mesa*, L. Valiente De Santos, I. Portales, B. Sobrino, A. Plata Ciezar, J.M. Reguera Iglesias, J.D. Colmenero Castillo (Malaga, ES)

Objectives: The aim of this study was to determine the incidence, clinical features, microbiologic findings and prognostic of cerebrospinal fluid (CSF) infection during external ventricular drainage (EVD).

Methods: We carried out a descriptive study of nosocomial EVD-associated CSF infections from January 2005 to November 2008 in Carlos Haya Hospital, Malaga, a tertiary hospital. In January 2008 we achieved clinical intervention for the management of EVD between Neurosurgery and Infectious Diseases Services with the objective to decrease the risk of infection.

Results: Nineteen patients had nosocomial EVD-associated CSF infections, with twenty three events of EVD infection, in 16 patients (4 event); two patients (2); and one patient (3). In 2005 were 7 cases; in 2006 (8); in 2007 (7); and in 2008 only 1 case. The incidence of infection in 2007 was 12.5% and 2008 was 2.27%. The mean age at the time of admission was 53.6±11.7 years (R: 31–72) and 63.2% were male. The latency of symptoms was 9.53±6.6 days (R=2–26). Open cranioatomy was achieved in 26.1% and 73.9% closed cranoatomy, with concomitant surgery in five cases. The drains were placed while the patient was in the emergency department in 89.5% of the cases. Prophylactic antibiotics were prescribed in 14 patients (63.6%). Seventeen patients (73.9%) were charged in ICU a mean time of 16±1 days (R=1–90), with EVD infection during the stay in 12 cases. Fourteen patients (61%) had crachecotony. The most frequent presenting symptoms and signs were fever (100%), headache (56.5%), altered mental status (52.2%), nausea and vomiting (30.4%) and meningesmus (56.5%). The infecting organisms isolated were Coagulase negative staphylococcus (11), Acinetobacter b. (6), Gram negative bacilli (5), Candida parasilis (1), Staph. aureus (1). Eighteen cultures were
monomicrobial and 4 polymicrobial. The mean duration of treatment was 29.83±17.1 days (r = 11−30), and intrathelial treatment in 16 cases. The removal of catheter was achieved in 91.3%, followed by placement of a new EVD in 78.3% and in 43.5% (10 patients) needed a VP-shunt. Seventeen patients (89.5%) had a excellent response to antimicrobial therapy, 2 patients (10.5%) had relapse and 4 patients (21.1%) had re-infections. The attributable mortality was 10.3% (2 cases).

Conclusion: The management of these patients should be standardised in order to reach better results and to reduce the risk infections at the Central Nervous System, especially after neurosurgery.

### P1454 Seasonal variation in the incidence of Gram-negative bacteria in intensive care units

**F. Schwab, P. Gastmeier, B. Schroeren-Boersch, E. Meyer** (Berlin, DE)

**Objective:** To look for an association of temperature changes and incidence densities of pathogens in intensive care units participating in SARI (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units).

**Methods:** We conducted a prospective unit based surveillance in 49 German ICUs from July 2000 to June 2008. Time-series analyses with autoregressive integrated moving average (ARIMA) models and dynamic regressor was used to estimate the association of the incidence densities of pathogens with seasons and temperature (winter: January to March, spring: April to June, summer: July to September and autumn: October to December).

**Results:** 49 ITS reported data on 1,292,600 patient days (pd) and 118,490 pathogens (63,293 Gram-positives and 55,198 Gram-negatives). Incidence densities i.e. the number of pathogens per 1000 pd of Gram-negative pathogens showed significant seasonal variation. They were also associated with the temperature 1 and/or 2 months before. Incidence densities for P. aeruginosa, E. coli and A. baumannii were significantly higher in autumn than in winter. Gram-positive pathogens were not associated with season.

Conclusion: Significant higher incidence densities of Gram-negative pathogens were observed during summer and autumn. These findings have implications for infection control and the choice of empiric antibiotic therapy.

### Pharmacokinetics and pharmacodynamics of β-lactams

**P1455 An open-label pharmacokinetic, safety and tolerability study of single-dose intravenous ceftaroline in subjects with end-stage renal disease on intermittent haemodialysis**

**T. Riccobene**, **A. Jakate, D. Rank, D. Thye** (Jersey City, Alameda, US)

**Objectives:** Cefuroxime (CPT) is a novel, parenteral, broad-spectrum cephalosporin exhibiting bactericidal activity against Gram-positive organisms, including methicillin-resistant Staphylococcus aureus and multidrug-resistant Streptococcus pneumoniae, as well as many Gram-negative pathogens. This open-label study evaluated safety, tolerability, and pharmacokinetics (PK) of an intravenous (IV) dose of CPT in subjects with end-stage renal disease (ESRD) on haemodialysis (HD) and in subjects with normal renal function.

**Methods:** 12 adult males (6 with ESRD and 6 with normal renal function [age, weight, and gender-matched]) received 400 mg CPT via a 1 hour IV infusion. ESRD subjects received 2 doses, one dose 4 hours before HD and one dose after HD, with at least a 7-day washout between doses. Blood, dialysate fluid, and urine (if possible from ESRD subjects), were obtained and analyzed for concentrations of CPT using a validated LC-MS/MS method. Adverse events (AEs) were monitored, physical examination findings, vital signs, clinical laboratory tests, and electrocardiograms (ECGs) were recorded at baseline and throughout the study.

**Results:** Plasma PK results in subjects with ESRD and normal renal function are summarised in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ESRD</th>
<th>Normal renal function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>17.5±3.75</td>
<td>29.1±8.49</td>
</tr>
<tr>
<td>AUC∞-0−t (µg.h/mL)</td>
<td>84.1±13.8</td>
<td>128.5±12.5</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>1.01±0.03</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>CL (mL/h)</td>
<td>4870.7±853.11</td>
<td>3139.87±321.89</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.01±0.03</td>
<td>1.0±0.0</td>
</tr>
</tbody>
</table>

The AUC of CPT was greater in subjects with ESRD dosed before or after HD (increased by 76% and 167%, respectively) relative to subjects with normal function. Mean recovery of CPT in dialysate during the 4 hour HD session was 76.5 mg (or 21.6% of the administered dose). Four AEs occurred in 3 subjects during the study (2 ESRD subjects, 1 subject with normal function). All AEs were considered not related to study drug, except for muscle spasms in a subject with normal function which was considered possibly related and occurred 6 days after drug administration. No serious AEs, discontinuations due to AEs, or clinically meaningful changes in laboratory parameters, ECGs, or vital signs were observed.

**Conclusions:** The PK parameters of CPT were altered in ESRD subjects relative to subjects with normal renal function resulting in greater systemic exposure. HD removed CPT to some extent. Study data will be used in population PK analyses and simulations to explore target attainment (% T>MIC) for different dose regimens for subjects receiving HD.

### P1456 A new semi-physiological absorption model to assess the pharmacodynamic profile of cefuroxime axetil using non-parametric and parametric population pharmacokinetics


**Objectives:** Cefuroxime axetil is widely used to treat respiratory tract infections. This study presents a new population pharmacokinetic (PK) model for the oral absorption of cefuroxime axetil using nonparametric and parametric population PK methodology. To develop a semi-physiological population PK model and evaluate the pharmacodynamic (PD) profile for cefuroxime axetil.

**Methods:** Twenty-four healthy volunteers received 250 mg oral cefuroxime after a standardised breakfast. LC-MS/MS was used for drug analysis, NONMEM and S-ADAPT (results reported) for parametric population PK and NPAQ for nonparametric population PK modeling. Monte Carlo simulations were used to predict the time of non-protein bound concentration above the MIC (T>MIC).

**Results:** A model with one disposition compartment, a saturable and time-dependent drug release from stomach and fast drug absorption...
from intestine yielded precise (r > 0.992) and unbiased curve fits and an excellent predictive performance. Apparent clearance was 21.7 L/h (19.8% CV) and volume of distribution 38.7 L (17.6%). Robust (≥90%) probabilities of target attainment (PTA) were achieved by 250 mg Q12h (Q8h) cefuroxime for MICs ≤0.375 mg/L (0.5 mg/L) for the bacteriostasis target fT>MIC ≥40% and for MICs ≥0.094 mg/L (0.375 mg/L) for the near-maximal killing target fT>MIC ≥65%. For the fT>MIC ≥40% target, PTAs for 250 mg cefuroxime Q12h were >97.8% against S. pyogenes and penicillin-susceptible S. pneumoniae. Cefuroxime 250 mg Q12h (Q8h) achieved PTAs below 73% (92%) against H. influenzae, M. catarrhalis, and penicillin-intermediate S. pneumoniae for susceptibility data from various countries.

Conclusion:
1. Depending on the MIC distribution, 250 mg oral cefuroxime Q8h instead of Q12h should be considered especially for more severe infections that require near-maximal killing by cefuroxime.
2. The proposed semi-physiological population PK model was robust and yielded excellent curve fits for the variable oral absorption of cefuroxime axetil.

Methods:
Non-proteinboundconcentrationaboveMICof
pharmacokineticsandMonteCarlosimulation. Wewas determined by HPLC and NONMEM was used for population pharmacokinetics and pharmacodynamics of cefazidime between CF-patients and healthy volunteers to identify optimal dosage regimens.

Objectives:
Premature mortality in patients with cystic fibrosis (CF) is predominantly (90%) caused by respiratory tract infections. Early efficacious treatment against Pseudomonas aeruginosa can postpone chronic lung infection by this pathogen in CF-patients. The pharmacokinetics of cefazidime in CF-patients has rarely been compared to healthy volunteers in the same study. To compare the population pharmacokinetics and pharmacodynamics of cefazidime between CF-patients and healthy volunteers and to identify optimal dosage regimens.

Methods:
We studied eight CF-patients (total body weight: 42.9±18.4 kg, average±SD) and seven healthy volunteers (66.2±4.9 kg). Dose: 2 g cefazidime as 5 min intravenous infusion. Cefazidime was determined by HPLC and NONMEM was used for population pharmacokinetics and Monte Carlo simulation. We used a target time of non-protein bound concentration above MIC of ≥65% which represents near-maximal kill.

Results:
Unscaled total clearance was 19% lower and volume of distribution was 36% lower in CF-patients. Allometric scaling by fat-free mass reduced the between subject variability by 32% for clearance and by 18–26% for volume of both peripheral compartments compared to linear scaling by WT. The standard cefazidime dosage regimen 2 g/70 kg WT q4h as 30 min infusion had robust (>90%) PTAs for MICs ≤1 mg/L in CF-patients and ≤3 mg/L in healthy volunteers. Alternative modes of administration achieved robust PTAs up to markedly higher MICs of ≤5–12 mg/L in CF-patients for 5 h-infusions of 2 g/70 kg WT q8 h and ≤12 mg/L for continuous infusion of 6 g/70 kg WT daily.

Conclusion:
Prolonged cefazidime infusion had PTA expectation values between 74% and 94% in CF-patients against P. aeruginosa based on susceptibility data from five European CF-clinics.

Methods:
Data for mecillinam concentrations after intravenous bolus injection of a 400, 800, and 1200 mg dose, respectively, in healthy volunteers were on file at LEO Pharma. 11 measurements of serum concentration within 8 hours from the injection were recorded in 6 individuals for the 800 mg as well as the 1200 mg dose. For the 400 mg dose, 9 measurements within 6 hours were recorded in 9 individuals. The NPAG program was used to fit a linear two-compartment model to each of the three data sets. Monte Carlo simulations of various dosage regimens were then made with the compartment model, and results were obtained on MIC breakpoints with respect to a Time>MIC of 40 and 50%, respectively, for several values of the probability of target attainment (PTA = 50, 90, 95%). The free fraction of mecillinam in serum was assumed to be 0.9.

Results:
After the parameter estimation process, the compartment model was able to reproduce the measured serum concentrations with good precision. Using the population PK parameter distributions derived from data on the 800 and 1200 mg dose, we simulated the mecillinam concentration in serum when receiving a 1000 mg dose TID. For a target Time>MIC of 40% (PTA=95%), the calculated MIC breakpoint was 2.0 mg/L. Based on the PK parameter values obtained from the data on a 400 mg dose, another dosage regimen, 500 mg QID, was simulated for which a MIC breakpoint of 1.15 mg/L was found (for a Time>MIC of 40%, PTA=95%).

Conclusion:
According to the simulation results, a dose of 500 mg QID i.v. would suffice for UTI caused by non-ESBL producing E. coli. For ESBL-producing E. coli, if clinical data allow, the dose should be increased to 1000 mg TID. The high dose is still manageable since mecillinam has low toxicity; a total dose of 60 mg/kg is tolerated in mature humans. The results suggest that a clinical breakpoint of 1 mg/L should be recommended for mecillinam.
Cefuroxime prophylaxis for elective orthopaedic surgery

J.D. Knudsen*, U. Urisonbarren, C. Jensen, R. Kristensen, J. Mouton (Hvidovre, DK; Nijmegen, NL)

Background: Cefuroxime is widely used for orthopaedic surgery prophylaxis, mainly with the aim of protecting against the methicillin susceptible S. aureus, MSSA. A standard dose of 1.5 g is often given together with the anaesthesia, app. 30 min before the surgical procedure starts in-dependent of patient age, gender, weight or body-mass index (BMI).

With the aim of evaluating the effectiveness of this used prophylaxis, 2–4 serum samples from patients were taken during the surgical procedures.

Materials and Methods: Serum samples from 77 patients were evaluated using multiple hollow fibers. In total, 40 female and 37 male, all admitted for elective orthopaedic procedures, and otherwise healthy.

All patients gave their written permission together with their data on all admitted for elective orthopaedic procedures, and otherwise healthy.

From the measured serum concentrations, the values for max concentration, C(0), the volume of distribution, Vd, and the serum half-life, t1/2, were calculated. From these actual values for the groups of females and males, the pharmacodynamic parameter of importance for cephalosporins, the T>MIC, were calculated for bacteria as MSSA.

Results: The median (range) for female/male: Age in years: 66 (25–86)/54 (22–76); weight in kg: 70 (45–131)/86 (60–120); and BMI in kg/m²: 26 (17–44)/29 (21–35).

The calculated values (95% c.i.) for female/male were: Vd in L: 17 (14–19)/17 (22–23); C(0) in mg/L: 114 (94–134)/102 (75–130); t1/2: 26 (17–44)/29 (21–35).

Significantly correlations were found between weight and Vd (p < 0.01) and BMI and Vd (p < 0.01) and a tendency to an inverse correlation between BMI and C(0) (p = 0.05), for the pooled population.

The parameter T>MIC for these patients, with a goal of 240 min above a MIC of 4 mg/L (equal to MSSA and T>MIC for 50% of t.i.d. dosing) was only achieved, if males were less than 110 kg, and with a BMI < 33 kg/m², but for all our female patients.

Conclusion: For patients with BMI above 33 kg/m² and weighting more than 110 kg the cefuroxime dose for orthopaedic prophylaxis may be insufficient.

Pharmacodynamically-linked variable for the combination of cefaroline plus Novexel104

G. Drusano*, M. Castanheira, W. Liu, B. van Scoy, G. Williams, I. Critchley, T. Riccobene, D. Thye, R. Jones, A. Louie (Albany, North Liberty, Alameda, USA)

Objectives: To evaluate the optimal administration schedule of the broad-spectrum β-lactamase inhibitor Novexel104 (NXL104) in combination with the MRSA- and Gram-negative-active drug cefaroline (CPT) against a strain of K. pneumoniae carrying KPC-2 in high copy number plus TEM-1 and level127 IPLC. In total, 40 female and 37 male, all admitted for elective orthopaedic procedures, and otherwise healthy.

Methods: We employed our hollow fiber infection model (HFIM). CPT was administered as 600 mg Q8h in all experiments. We conducted 3 experiments. Two were dose ranging (DR) with NXL104 administered at different dose levels in continuous infusion (CI). The third was a fractionation experiment in which the same daily AUC of NXL104 was administered as a CI on Q24h, Q12h, and Q8 h schedules. Samples were taken for pharmacokinetics and for total and resistant organism counts. Resistant organisms were quantitated on plates containing 4 mg/L NXL104 plus 3×baseline MIC of CPT (MIC with 4 mg of NXL104). MIC values were determined by CLSI methods. CPT and NXL104 were measured in Mueller-Hinton II broth (medium of HF experiments) by LC/MS/MS. Target organism density was 3×10⁷. All experiments were carried out for >10 days.

Results: The MIC for CPT in the presence of 4 mg/L of NXL 104 was 0.75 mg/L. For CPT and NXL104 alone, MIC values were 1024 and >64 mg/L, respectively. The first DR experiment examined CI concentrations of NXL104 from 2 through 8 mg/L. NXL104 at 2 and 4 mg/L allowed emergence of resistance after 1 and 6 days, respectively. Six mg/L had resistance failure at the very end (day 10). Eight mg/L had no resistance and provided multi-log cell kill for the whole 10 days. In the second DR experiment, 6 mg/L did not have resistance emergence, along with higher concentrations (8, 10, 12 mg/L). Microbiological effect was optimal at 10–12 mg/L. In the third experiment, we fractionated the exposure for 8 mg/L (8 mg/L CI = AUC 192 mg*h/L; AUC 192 once; AUC 96 Q12h; AUC 64 Q8h). Q24 administration failed with resistance at day 3. All others suppressed resistance. Microbiological effect was optimal for 8 hourly and continuous infusion. In all experiments, CPT alone demonstrated no microbiological activity against this strain.

Conclusion: Resistance suppression for NXL104 administration in the background of CPT 600 mg 8 hourly is linked to Time>$Threshold. The linkage for effect is, therefore, the same for both the β-lactam and the β-lactamase inhibitor, implying that they can be administered together on the same schedule.
Objective: NXL104 is a novel β-lactamase inhibitor undergoing Phase II clinical evaluation in combination with ceftriaxone (CAZ). CAZ pharmacodynamics (PD) are Time>MIC dependent, but little is known about the relationship of pharmacokinetics (PK)/PD for the combination. The aim of this study was to determine the importance of NXL104 PK on the PD of CAZ+NXL104 combinations.

Methods: Exponentially growing Enterobacter cloacae 293HT96 (Ecl) ( Amp(C), Klebsiella pneumoniae (Kpn) Tunisie C4 (CTX-M-15), Kpn 181 and Kpn 236 (SHV-5, TEM-1) were exposed to various dosing regimens of the combination in a hollow-fiber infection model: (1) CAZ+NXL104 continuous infusion; and (2) CAZ+NXL104 human-like profile (mimicking a biexponential profile following a single 30 min intravenous infusion in humans). CAZ was held constant at 16 mg/L throughout the assay. NXL104 was added so as to have the same total exposure in both regimens, but with different concentration-time curves. Samples were taken at different time points for determination of viable bacterial counts and CAZ and NXL104 concentrations.

Results: The combination CAZ+NXL104 was rapidly cidal against Ecl, Kpn C4, Kpn 181 and Kpn 236, reducing the bacterial count by 3 log10 within 4h. Growth of the four strains was fully suppressed throughout the assay. NXL104 was added so as to have the same total exposure in both regimens, but with different concentration-time curves. Samples were taken at different time points for determination of viable bacterial counts and CAZ and NXL104 concentrations.

Conclusions: Findings qualitatively support maintenance of a critical NXL104 concentration as one of the important PD factors for the CAZ+NXL104 combination under these experimental conditions. This critical concentration of inhibitor is necessary to sufficiently suppress β-lactamase activity.

Objective: Methicillin-susceptible Staphylococcus aureus (MSSA) is frequently involved in skin and skin structure infections (SSSIs). Moxifloxacin (MXF), ceftriaxone (CRO) and amoxicillin/clavulanic acid (AMX/CLA) have been approved for this indication. This study compares the bactericidal activities of MXF, CRO and AMX/CLA against a clinical MSSA isolate in a hollow fibre (HF) pharmacokinetic/pharmacodynamic (PK/PD) model.

Methods: MICs were determined by the broth microdilution method according to CLSI guidelines. A recent clinical isolate, MSSA strain HF1012717, was exposed to MXF (400 μg/mL; Cmax 2.0 mg/L, T1/2 12 h; MIC = 0.03 mg/L), CRO (2000 μg/mL; Cmax 25.7 mg/L, T1/2 7.5 h; MIC = 4 mg/L) and AMX/CLA (1125 mg b.i.d.; Cmax 13.5/1.7 mg/L, T1/2 1.3 h; MIC = 2 mg/L) in the PK/PD model. These concentration-time profiles are equivalent to human free drug serum pharmacokinetics. For the PK/PD model, an initial inoculum of 10^8 CFU/mL was applied and all experiments were performed, in triplicate, over 24h. Antimicrobial concentrations were determined by bioassay. Antimicrobial effect was measured as the time to achieve a 3-log10 unit reduction (bactericidal kill) and the log10 unit reduction in viable counts per mL after 24h. MXF achieved a 3-log10 unit kill at 1.5–2.5 h. When CRO and AMX/CLA pharmacokinetics were simulated, a 3-log10 unit kill was seen at 7.5–8.5 h. After 24 h, the MXF regimen led to a reduction of about 3.4 log10 units whereas the CRO and AMX/CLA regimens resulted in reductions of 3.4 log10 units and 3.0 log10 units, respectively.

After 24 h, neither significant re-growth nor elevation in the MICs of the isolated colonies had occurred under any regimen.

Conclusions: Simulated human pharmacokinetics of MXF, CRO and AMX/CLA showed bactericidal activity against an MSSA isolate when tested in an HF PK/PD model. MXF was significantly more effective than CRO and AMX/CLA with regard to time to a 3-log10 unit reduction in bacterial counts and total bactericidal activity after 24h.

Objective: Extended daily dialysis (EDD) is an increasingly popular mode of renal replacement therapy in the ICU (intensive care unit) as it combines the advantages of intermittent haemodialysis (IHD) and continuous renal replacement therapy (CRRT), i.e. excellent detoxification accompanied by cardiovascular tolerability. The aim of this study was to evaluate pharmacokinetics (PK) of ertapenem, the newest carbapenem with once-daily dosing, in critically ill patients with anuric acute renal failure (ARF) undergoing EDD.

Methods: In a single-centre, prospective, open-label study six ICU patients with ARF undergoing EDD were treated with 1 g ertapenem given as a single intravenous dose. EDD was performed using a high-flux dialyzer (polysulphone, 1.3 m2). Blood and dialysate flow were 160 mL/min, and the length of treatment was 480 min. Plasma samples were collected at different time points up to 24 h after medication. Drug concentrations were determined by a validated LC-MS method. Free drug concentrations were estimated using a two-class binding site equation.

Results: After a single dose of 1000 mg free ertapenem, protein-unbound plasma concentrations exceeded a MIC90 value of 2 mg/L for >20 h after dosing. The clearance of the tested dialyzer was 38.5±14.2 mL/min.

Conclusions: In contrast to patients undergoing regular IHD, in which a dose reduction is required, our data suggest that in patients treated with EDD a standard dose of ertapenem (1 g/day), i.e. dose for patients without renal failure, is required to maintain adequate plasma drug levels.

Objective: 14.2 mL/min. Increased glomerular filtration rate associated with low imipenem blood concentrations in febrile neutropenic cancer patients.

Methods: IMP therapy courses in febrile neutropenic patients were retrospectively studied. The renal function was evaluated by the calculated GFR (Cockroft-Gault). Trough blood concentrations (HPLC) were interpreted as low if <MIC or MIC/2 of the most common bacteria in febrile neutropenia (1 mg/L, EUCAST data). The IMP dose adjustment to the renal function was evaluated by the daily dose per 100 mL/min GFR (2 to 4 g/d per 100 mL/min GFR are recommended in febrile neutropenic patients. This study aimed at evaluating the relationship between GFR, IMP doses and blood concentrations in febrile neutropenia.

Methods: IMP therapy courses in febrile neutropenic patients were retrospectively studied. The renal function was evaluated by the calculated GFR (Cockroft-Gault). Trough blood concentrations (HPLC) were interpreted as low if <MIC or MIC/2 of the most common bacteria in febrile neutropenia (1 mg/L, EUCAST data). The IMP dose adjustment to the renal function was evaluated by the daily dose per 100 mL/min GFR (2 to 4 g/d per 100 mL/min GFR are recommended in febrile neutropenia).

Results: 50 febrile neutropenic episodes were studied (18 microbiologically and 20 clinically documented infections, 12 FUO). The median GFR was 82 mL/min (range: 31–230). Median IMP blood trough concentrations measured at a median of 2 days (1–9) after start of therapy were 0.8 mg/L (0.1–5). IMP daily dose adjusted to GFR and trough concentrations displayed a linear correlation.
Resistance to non-β-lactams in Enterobacteriaceae

M.E. Cuno*, J. Calco, J. Agüero, L. Martinez-Martínez (Santander, ES)

Objectives: Plasmid-mediated quinolone resistance (PMQR) caused by qnr, qepA and/or aac(6’)-Ib-cr genes have often been investigated in enterobacteria with concrete resistance phenotypes or in particular species. In this study, the presence of PMQR genes in enterobacteria consecutively isolated from blood cultures was evaluated using a multiplex PCR.

Methods: Three hundred and forty-two Enterobacteriaceae (1/patient) consecutively isolated from blood cultures (January 2007-March 2008) were studied. Identification and susceptibility testing were performed with the MicroScan WalkAway system (Dade Behring). Isolates containing PMQR genes were typed by REP-PCR and the MICs of nalidixic acid (NAL), ciprofloxacin (CIP) and levofloxacin (LEV) against them were determined by Etest. qnrA, qnrB, qnrS, qepA and aac(6’)-Ib-cr were detected by a multiplex PCR (degenerated primers for amplifying 20 qnrB variants were used). Positive reactions were confirmed by sequencing (both strands) of amplicons obtained with other primers amplifying the entire, or almost entire, gene.

Results: We tested 180 E. coli, 44 K. pneumoniae, 31 E. cloacae, 24 S. marcescens, 17 P. mirabilis, 13 K. oxytoca, 10 C. freundii, 8 E. aerogenes and 15 other species. The multiplex PCR correctly detected PMQR genes of positive controls, individually and in all possible combinations. PMQR genes were overall detected in 24 (7%) isolates. qnrB, qnrS and aac(6’)-Ib-cr were detected in five (1.5%), 13 (3.8%) and seven (2%) isolates, respectively. One E. coli contained both qnrS1 and aac(6’)-Ib-cr. No isolates carried qepA or qepA. Four of 5 qnrB variants were new alleles detected in 4 clonally unrelated C. freundii; the remaining case corresponded to qnrB1 in an E. cloacae. qnrS genes corresponded to 12 qnrS1 (3 in 3 clones of E. coli, 6 in 3 clones of E. cloacae, 1 isolate of K. pneumoniae) and 1 qnrS2 (in a K. pneumoniae strain clonally unrelated to the qnrS1-positive strain). aac(6’)-Ib-cr was detected in 6 E. coli (4 clones) and 1 E. aerogenes. In the 24 isolates with PMQR genes, resistance (CLSI breakpoints) to NAL, CIP and LEV were 75%, 46% and 42%, respectively.

Conclusion: PMQR genes were successfully detected with the multiplex PCR developed in this study. qnrS variants were the more common PMQR genes detected, followed by aac(6’)-Ib-cr and qnrB alleles. A significant proportion of strains with these genes are susceptible to both nalidixic acid and fluoroquinolones.
Conclusion: We firstly describe the qnrS1 gene on an IncN plasmid of a Salmonella Enteritidis strain, a serotype commonly implicated in human infections. Despite the presence of this gene on a conjugative plasmid, a low prevalence of qnr genes was observed in Portugal.

**P1470** Dissemination of a small ColE1-like plasmid harbouring qnrB19 among commensal Escherichia coli, Bolivia

L. Pallecchi*, E. Riccobono, A. Mantella, S. Sennati, F. Bartalesi, G. Lenti, C. Trigoso, A. Bartoloni, G.M. Rossolini (Sienna, Florence, IT; La Paz, BO)

Background: In 2005, qnrB19 was detected in three clonally unrelated Escherichia coli isolates from the commensal microbiota of healthy children living in two urban areas of Bolivia. Here we investigated the qnrB19-harbouring plasmids from those isolates.

Methods: Electroporation was used for plasmid transfer in E. coli HB101. Susceptibility tests were performed by the microdilution method, as recommended by CLSI. Plasmid analysis was carried out by RFLP (Restriction Fragments Length Polymorphism), PCR replicon typing (Carattoli et al.), and sequence analysis.

Results: The three qnrB19-harbouring plasmids were transferred in E. coli HB101 using nalidixic acid 8 μg/ml for selection of transformants. All plasmids showed the same RFLP pattern after digestion with SacII or HindIII or EcoRI, and an estimated size of 3.0 Kb. Replicon types could not be identified using the PCR method. Sequence analysis of the qnrB19-harbouring plasmids showed that qnrB19 was located on ColE1-like plasmids. The genetic regions flanking qnrB19 showed 100% homology with those reported in the qnrB19-harbouring transposons Tn5387 from USA (244 bp upstream and 225 bp downstream) and Tn1721 from Colombia (155 bp upstream and 225 bp downstream). However, neither ISEcp1 nor other putative transposases were identified in the qnrB19-harbouring plasmids from Bolivia.

Conclusions: We observed the dissemination of a small qnrB19-harbouring ColE1-like plasmid among commensal E. coli from healthy children living in Bolivia. The genetic environment of qnrB19 was found to be different from that of the previously described qnrB19 genes.

**P1471** Prevalence of qnr genes in nalidixic acid resistant, ciprofloxacin sensitive Escherichia coli and Klebsiella pneumoniae clinical isolates from a university general hospital, Athens, Greece

N. Mitchell, I. Galani, M. Souli, H. Giamparellou* (Athens, GR)

Objectives: To determine the prevalence of plasmid-mediated quinolone resistance (qnr) genes in nalidixic acid resistant (NR), ciprofloxacin sensitive (CS) E. coli and K. pneumoniae clinical isolates.

Methods: During a five month period (February to June 2008) 231 E. coli and 231 K. pneumoniae isolates were collected from separate inpatients and outpatients of the Attikon University General Hospital. Of these samples, 13 E. coli and 11 K. pneumoniae isolates had the above phenotype. Ciprofloxacin MIC values ranged from ≤ 0.125 to 1 mg/L for the E. coli isolates and <0.125 to 0.5 mg/L for the K. pneumoniae isolates. Isolates were screened for the presence of genes qnrA, qnrB and qnrS by multiplex PCR, using universal primers for each gene amplifying all related alleles. The qnr positive isolates were confirmed by genomic sequencing. The qnr positive isolates were additionally tested for extended-spectrum β-lactamases (ESBL) and metallo-β-lactamases (MBL).

Results: One E. coli (1/13, 7.7%) was positive for qnrB and one K. pneumoniae (1/11, 9.1%) was positive for qnrA. No isolates from those collected were positive for qnrS. Both the qnrA positive and the qnrB positive isolates were ESBL negative, MBL positive, carrying the blaVIM-1 gene.

Conclusions: This is the first report of a qnrA positive K. pneumoniae and qnrB positive E. coli with concurrent blaVIM-1 genes. Among NR, CS E. coli and K. pneumoniae clinical isolates collected during this five month time period, the qnr gene prevalence rate detected was 7.7% and 9.1% respectively.

**P1472** Association of DNA gyrase mutations and qnrS1 gene in quinolone-resistant E. coli isolated from food animal, in Italy

C. Forcella*, A. Alessiani, E. Di Giannatale, K. Zilli, M. Perilli, G. Celenza, G. Amicosante (L’Aquila, Teramo, IT)

Objectives: Quinolones recognize as targets both DNA gyrase and topoisomerase IV; thus the most common mechanism of resistance is originated by altered domains in these enzyme active sites that can reduce or block the drug binding capacity. The aim of the study was to investigate the quinolone resistance in E. coli collected from food animal in an Italian region.

Methods: Fifty nine E. coli were isolated from food animal (chicken, rabbit, ship, bovine) during 2000–2007 period in the Istituto Zooprofilattico Sperimentale Abruzzo e Molise (IZSAM), Italy. Molecular analysis was performed by colony hybridisation using specific probes for integrase (IntI1) genes, by PCR, sequencing and PFGE analysis.

Results: Among the screened isolates, 44/59 E. coli were found to be resistant to nalidixic acid but susceptible to ciprofloxacin; 4/59 isolates were resistant to ciprofloxacin and nalidixic acid susceptible; 11/59 isolates were resistant to ciprofloxacin and nalidixic acid. Quinolone Resistant Determinant Region (QDR) of gyrA and gyrB of 59 E. coli were amplified and sequenced in order to verify the presence of mutations. Twenty eight isolates showed a single mutation in gyrA at position 83 where a residue of serine was changed in leucine (27/28 isolates) or alanine (one isolate). One isolates showed an additional mutation Asp87Ala. Mutations in gyrB were found only in three E. coli (3/59) The mutations were as follows: Gln434His, Lys447Arg (in association with Ser83Leu) and Gly435Val (in association with Ser83Leu). Large plasmids sized 100 Kb were detected in 16 out 59 isolates. All E. coli were also screened for the presence of qnr elements. Only one isolate, with Ser83Leu mutation, showed the presence of qnrS1 element. The PFGE analysis of 59 E. coli showed no relatedness.

Conclusions: In this study we demonstrated that quinolone resistance is mediated by chromosomal mutations in bacterial topoisomerase and qnr genes responsible for plasmid-borne quinolone resistance in E. coli isolated from food animal. Resistance to quinolones in zootonically transmitted enterobacteria is an almost inevitable consequence of the use of these molecules in food animal production.

**P1473** Decreased quinolone susceptibility in high percentage of Enterobacter cloacae clinical isolates caused only by Qnr determinants

X. Zhao, X. Xu, D. Zhu, X. Ye, M. Wang* (Shanghai, CN)

Objectives: To investigate the prevalence of Qnr and other plasmid-mediated quinolone resistance (PMQR) determinants in Enterobacter cloacae clinical isolates, and to determine the role of Qnr and/or target mutations in the development of quinolone resistance.

Methods: The presence of qnrA, qnrB, qnrS, aac(6’)-Ib-cr and qepA genes was evaluated by PCR in 101 consecutive strains of E. cloacae, including quinolone-resistant and -susceptible strains, isolated at a university hospital in Shanghai from January to December 2005. ESBLs and plasmid-mediated AmpC β-lactamases were identified, and their genotypes were determined. Mutations in the quinolone resistance-determining region (QRDR) of gyrA and parC were determined by PCR amplification and DNA sequencing for PMQR positive strains.

Results: Of 101 E. cloacae strains, 33 (32.7%) and 4 (4%) were resistant and intermediate to ciprofloxacin, respectively, and 52 (51.5%) produced ESBL and/or acquired AmpC β-lactamases. PMQR determinants were present in 42 (41.6%) isolates with qnrA and aac(6’)-Ib-cr detected alone or in combination in 39 (38.6%) and 7 (6.9%) strains, respectively. qepA was not detected. qnr genes included 19 qnrA, 18 qnrB and 3 qnrS; one strain was positive for both qnrB and qnrS. qnr genes were present in 48.4% (16/33) and 32.8% (21/64) of ciprofloxacin-resistant and -susceptible strains, respectively (Pearson Chi-square test, P = 0.22), indicating that the prevalence of qnr genes was similar in the
ciprofloxacin-resistant and susceptible groups. No amino acid change in QRDRs was found in any of the 21 qnr positive, but ciprofloxacin-susceptible strains of which 20 had ciprofloxacin MICs of 0.06 to 0.25 μg/ml and one an MIC of 1 μg/ml, indicating that decreased quinolone susceptibility in those clinical strains may be caused by Qnr, but not by target mutations. Two or more amino acid substitutions in GyrA and/or ParC were detected in 15 of 16 qnr positive strains with ciprofloxacin MICs 8 to ≥ 32 μg/ml.

Conclusion: The presence of qnr genes in ciprofloxacin-susceptible strains was similar to that in ciprofloxacin-resistant strains. Qnr may be present prior to target mutations in some clinical strains with decreased quinolone susceptibility.

**Materials and Methods**: The study included 114 single E. coli isolates from 4 Greek tertiary care hospitals. All isolates were identified and tested for antibiotic susceptibility with the VITEK 2 automated system (bioMérieux, Marcy l’Etoile, France). All isolates were highly CIP-resistant (MIC ≥ 4). Resistance by target modification was screened by PCR amplification and sequencing of the quinolone resistance determining regions (QRDRs) of gyrA and parC genes. Synergy experiments were also performed using ciprofloxacin and the proton-gradient dependent efflux pump inhibitor carbonyl-cyanide-m-chlorophenoxy-hydrazon (CCCP), to check the contribution of efflux pumps. The presence of qnrA, qnrB and qnrS was also screened by PCR.

**Results**: All high-level CIP-resistant E. coli had two mutations in gyrA in combination with mutations in parC genes. Mutations in parC were only found in combination with gyrA mutations. The mutations found for gyrA were S83L, D87N and D87Y and for parC were S80R, S80I, E84V, E84K and E84G. 111 isolates had only the gyrA mutations. 17 isolates had double mutations both in gyrA and parC. 84 had double mutation in gyrA and a single mutation in parC. Contribution of efflux pump mechanism was not determined in the isolates of the study. No qnrA or qnrB gene was detected, whereas 11 qnrS-positive E. coli isolates were found by sequencing of the amplicons to carry the qnrS1 allele. PFGE was performed in qnrS1 isolates that were found to belong in distinct genotypes.

**Conclusions**: Target mutation in QRDRs was the most prevalent mechanism of quinolone resistance in Greek CIP-resistant E. coli isolates. Transferrable resistance by target protection or enzymatic modification was less common (9.6%). qnr genes seem to be common in ciprofloxacin-resistant clinical E. coli isolates and may contribute to the alarming rates of quinolone resistance in Greece.

**Objective**: Quinolone resistance is relatively common among Greek Escherichia coli isolates. The underlying quinolone resistance mechanisms were investigated in 114 ciprofloxacin (CIP) resistant E. coli isolates from individual patients in 4 Greek tertiary care hospitals.

**Methods** and **Results**: The study included 114 single E. coli isolates from 4 Greek tertiary care hospitals. All isolates were identified and tested for antibiotic susceptibility with the VITEK 2 automated system (bioMérieux, Marcy l’Etoile, France). All isolates were highly CIP-resistant (MIC ≥ 4). Resistance by target modification was screened by PCR amplification and sequencing of the quinolone resistance determining regions (QRDRs) of gyrA and parC genes. Synergy experiments were also performed using ciprofloxacin and the proton-gradient dependent efflux pump inhibitor carbonyl-cyanide-m-chlorophenoxy-hydrazon (CCCP), to check the contribution of efflux pumps. The presence of qnrA, qnrB and qnrS was also screened by PCR.

**Results**: All high-level CIP-resistant E. coli had two mutations in gyrA in combination with mutations in parC genes. Mutations in parC were only found in combination with gyrA mutations. The mutations found for gyrA were S83L, D87N and D87Y and for parC were S80R, S80I, E84V, E84K and E84G. 111 isolates had only the gyrA mutations. 17 isolates had double mutations both in gyrA and parC. 84 had double mutation in gyrA and a single mutation in parC. Contribution of efflux pump mechanism was not determined in the isolates of the study. No qnrA or qnrB gene was detected, whereas 11 qnrS-positive E. coli isolates were found by sequencing of the amplicons to carry the qnrS1 allele. PFGE was performed in qnrS1 isolates that were found to belong in distinct genotypes.

**Conclusions**: Target mutation in QRDRs was the most prevalent mechanism of quinolone resistance in Greek CIP-resistant E. coli isolates. Transferrable resistance by target protection or enzymatic modification was less common (9.6%). qnr genes seem to be common in ciprofloxacin-resistant clinical E. coli isolates and may contribute to the alarming rates of quinolone resistance in Greece.
metronidazol during seven days. Two faecal S. typhimurium isolates were obtained before (Se6) and after (Se20) treatment, showing Se20 resistance to quinolones and tobramycin. The clonal relationship among both isolates was examined by PFGE. Antimicrobial susceptibility was determined by disk diffusion and agar dilution methods. Presence of the following genes was studied by PCR and sequencing: qnrA, qnrB, qnrS, qepA; tetA(3′)–E, aph(3′)–Ia, aph(3′)–Ia, aac(6′)–Ib, adaA, strA–strB; sul1, sul2, sul3; class 1, 2, and 3 integrase genes, and qacDE1–sul1 region of class 1 integron. Mutations in gyrA and parC genes were studied by PCR and sequencing. Mating experiments to Escherichia coli recipient strain was assessed.

Results: Both S. typhimurium strains presented indistinguishable PFGE patterns and were resistant to tetracycline, streptomycin and sulfonamides, but susceptible to all β-lactams and gentamicin. The MIC values (mg/L) for Se6/Se20 were as follows: nalidixic acid (16/32), ciprofloxacin (0.5/8), norfloxacin (4/8), kanamycin (4/64), tobramycin (1/32), amikacin (4/16) and trimethoprim (1/128). S. typhimurium Se6 and Se20 presented the sul2–strA–strB structure, tetA(3′) and qnrS1 genes, but quinolone and tobramycin resistance found in Se20 was due to the presence of aac(6′)–Ib–cr gene and the Ser83Tyr substitution in GyrA. This strain also presented one defective class 1 integron lacking qacDE1–sul1, which included the trimethoprim resistance dfrA17 gene cassette downstream of intI1 gene. The two types of E. coli transconjugants obtained from Se20 (Se20A/Se20L) acquired qnrS1, sul2 and intI1–dfrA17 genes, but not strA–strB or tetA(3′). In addition, the Se20A included the aac(6′)–Ib–cr gene, but not the Se20L, being their different MICs (mg/L) as follows: ciprofloxacin (4/1), kanamycin (64/4), tobramycin (32/1), and amikacin (8/4).

Conclusions: First report of in vivo selection of aac(6′)–Ib–cr gene and Ser83Tyr change in GyrA in a qnrS1-positive S. typhimurium strain after plasmid-mediated treatment; in vitro transfer of both plasmid-mediated quinolone resistance genes is demonstrated.

P1479 Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum β-lactamase in clinical isolates from Korea


Objectives: The aims of this study were to investigate the prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum β-lactamase (ESBL) in clinical isolates from Korea.

Methods: A total of 347 non-duplicated isolates of Enterobacteriaceae and 200 non-duplicated isolates of Pseudomonas aeruginosa in 2006 were collected from various clinical specimens in two hospitals. The qnr determinant screening was carried out by PCR amplification of qnr genes, and all positive results were confirmed by direct sequencing of the PCR products. We determined the presence of ambler class A β-lactamase and AmpC β-lactamase genes for qnr-positive strains to investigate the association with ESBL.

Results: The qnr gene was detected in 47 of the 347 clinical Enterobacteriaceae isolates. No qnr gene was detected in 200 P. aeruginosa strains. Among 47 qnr-positive strains, K. pneumoniae (N = 30) was the most common, followed by E. coli (N = 7), E. cloacae (N = 5), and others (N = 5). These were finally identified as new qnrA1 like (N = 6), eight qnrB subtype (N = 40) including new qnrB4 like (N = 29) and qnrB12 like (N = 1), and qnrS1 (N = 1). The antimicrobial-resistance rates of qnr-positive strains to ciprofloxacin, levofloxacin, norfloxacin, nalidixic acid, and moxifloxacin were 51.1%, 46.8%, 46.8%, 74.5%, and 53.2%, respectively. The qnr-positive strains showed also high positive rates of ESBL such as TEM (N = 16), SHV (N = 29), CTM-M (N = 15), and DHA (N = 23).

Conclusion: The qnr genes were highly prevalent in various clinical isolates, mainly the qnrB subtypes. The qnr-positive strains were closely associated with diverse ambler class A and AmpC β-lactamases.

P1478 A high-level fluoroquinolone-resistant Salmonella Typhimurium strain shows decreased invasion ability. Is there any regulatory link?

A. Fabrega*, L. du Merle, C. Le Bouguenec, J. Vila (Barcelona, ES; Paris, FR)

Objective: The main objective was to determine a possible link between ciprofloxacin-resistance in Salmonella Typhimurium and a loss of expression of virulence genes, such as those encoding invasion proteins. This link could justify why nalidixic acid resistance has steadily been increasing during the last years, whereas ciprofloxacin resistance has remained stable.

Methods: A high-level ciprofloxacin resistant mutant (strain 50–64) was obtained in vitro from a susceptible clinical isolate. A reverted phenotype of 50–64 could be obtained (strain 50–rev). Sequencing of the genes encoding the target proteins as well as those encoding a multiple antibiotic resistance (MAR) phenotype was performed. Analysis of nalidixic acid, norfloxacin and ciprofloxacin resistance was checked in the presence and absence of PAN (an efflux pump inhibitor). A Western Blot methodology was carried out using antibodies against AcrB and ToIC. A full-genome microarray analysis was performed. The invasion ability was determined using the gentamicin protection assay.

Results: The sequencing analysis showed that 50–64 acquired 3 mutations (2 in gyrA (G81C and D87G) and 1 in parE (E470K)). No other mutation was found in any regulatory protein (AcrR, MarR, SoxR, RamR). 50–64 achieved a MIC of ciprofloxacin of 64 mg/L, which reverted to 1.5 in 50-rev. In the presence of PAN, both strains showed a similar MIC of ciprofloxacin (1 mg/L). Western Blot analysis showed overexpression of AcrB and ToIC in 50–64 that decreased to nearly the wild-type levels in 50-rev. The invasion results showed a decrease in the percentage of invasion from 11.1 to 0.2% in 50–64, whereas a significant reversion could not be detected in 50-rev (0.7%). The microarray results demonstrated a significant decrease in 50–64 of the whole SPI-1 (Salmonella Pathogenicity Island) and all the promoters that encode flagellum assembly and function. However, only a partial reversion could be detected in 50-rev.

Conclusion: High-level quinolone resistance not only depends on mutations within the target genes but also on the overexpression of the main efflux pump, AcrAB. This ciprofloxacin resistance can be reverted to a decreased-susceptibility phenotype. No mutation could be found in AcrR, MarR, SoxR and RamR, another regulatory loci must be implicated to justify the increased expression of AcrAB. This regulation could be linked with the decreased expression of the SPI-1 and flagellum operons that may lead to decreased invasion ability.
Results: Sequencing results showed that Y-64 had acquired four different point mutations within the QRDRs: one in gyrA (D87Y), one in gyrB (S464L), and two in parC (S84R and A85E). Y-rev did not show any reversion of the resistance phenotype. In the presence of PAN, only the MIC of nalidixic acid decreased 64-fold in Y-64, whereas the MICs of ciprofloxacin and norfloxacin remained the same. SDS-PAGE analysis showed overexpression of AcrA and AcrB in Y-64. The RT-PCR assays showed a decreased expression of yadA whereas marA mRNA increased significantly. The gentamicin protection assay indicated a decrease in the percentage of invasion, from 6.9% in Y-wt to 0.2 in Y-64.

Conclusion: This high-level ciprofloxacin resistance phenotype has been generated from the acquisition of four mutations within the QRDRs. In addition, the overexpression of AcrAB is also present in Y-64. However, it does not seem to affect the fluoroquinolones MICs. AcrAB may only be important for nalidixic acid resistance. This is the first report of a marA homolog found in Y enteroxolitica which is, in addition, overexpressed in a quinolone-resistant strain, which may explain the AcrAB overexpression detected. Furthermore, the decrease in the percentage of invasion, as well as the decrease in yadA expression, suggests a link between the regulatory networks behind these two phenotypes.

Prevalence of hypermutability in clinical isolates of Klebsiella pneumoniae and its role in ciprofloxacin resistance
S. Aastithan*, G.L. French (London, UK)

Objectives: To investigate the prevalence of hypermutability in clinical isolates of Klebsiella pneumoniae, and its possible role in the accumulation of mutations in the quinolone-resistance-determining region (QRDR) and hence in ciprofloxacin resistance.

Methods: Sixty-four distinct clinical isolates of K. pneumoniae with widely differing ciprofloxacin MICs (range 0.016 to =512 mg/L) and known gyrA and parC QRDR sequences, were investigated for high frequency of mutations to rifampicin resistance using selective media containing 100 mg/L rifampicin. Twelve selected isolates with multiple, single or no QRDR mutations were also assessed for high frequency of mutation (or further mutation) to ciprofloxacin resistance, using selective media containing ciprofloxacin concentrations at 4 × the MICs of the respective isolates.

Results: The rifampicin study identified three hypermutable isolates amongst the 64 tested (>5%); one was ciprofloxacin susceptible (1 of 12 such isolates), one ciprofloxacin resistant (1 of 28) and one high-level ciprofloxacin resistant (1 of 24). There was no association between ciprofloxacin MIC or gyrA and parC mutations and hypermutability. The ciprofloxacin study identified only two of the hypermutant isolates found by the rifampicin study (one was the ciprofloxacin susceptible isolate with no mutations in the gyrA parC QRDR; the other was the highly ciprofloxacin resistant isolate with double mutations in gyrA and a single mutation in parC QRDR). The ciprofloxacin study identified no other hypermutable isolates.

Conclusions: Hypermutation is uncommon in clinical isolates of K. pneumoniae and occurs randomly amongst ciprofloxacin resistant and susceptible isolates and in those with both no and multiple mutations in gyrA and parC. This suggests that hypermutation contributes to neither the accumulation of mutations in the QRDR nor directly to ciprofloxacin resistance.

Study of fluoroquinolone resistance mechanisms among extended-spectrum β-lactamase-producing E. coli urinary isolates in Madrid, Spain

Objectives: During 2005 a total of 191 ESBL-producing E. coli strains were isolated from urine in our hospital and a high percentage of ciprofloxacin (Cip) resistance was observed (75%) corresponding to 141 strains. We studied the mechanism accounting for fluoroquinolone resistance in these isolates.

Methods: Of the 141 Cip-resistant strains 30 isolates were chosen to represent the full range of Cip resistance and were classified in three groups according to the Cip MIC: Group 1 MIC range 4–8 mg/L, Group 2 MIC range 16–64 mg/L and Group 3 MIC >128 mg/L. In the selected isolates sequencing of the QRDR of the gyrA and parC genes was performed. All 191 ESBL-producing strains were screened for the presence of the qnrA, qnrB and qnrS genes by multiplex-PCR. Mating assays with E. coli K-12 as recipient strain were attempted to determine the transferability of qnr genes.

Results: Distribution of gyrA and parC sequences is displayed in the Table.

<table>
<thead>
<tr>
<th>Group (MIC range, mg/L)</th>
<th>No. of strains</th>
<th>gyrA</th>
<th>parC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ser83</td>
<td>Asp87</td>
</tr>
<tr>
<td>1 (4–8)</td>
<td>5</td>
<td>Leu</td>
<td>Asn</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Leu</td>
<td>Gly</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Leu</td>
<td>Asn</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Leu</td>
<td>Tyr</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Leu</td>
<td>WT</td>
</tr>
<tr>
<td>2 (16–64)</td>
<td>4</td>
<td>Leu</td>
<td>Asn</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Leu</td>
<td>Asn</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Leu</td>
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<td></td>
<td>1</td>
<td>Leu</td>
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</tr>
<tr>
<td></td>
<td>1</td>
<td>WT</td>
<td>Asn</td>
</tr>
<tr>
<td>3 (&gt;128)</td>
<td>5</td>
<td>Leu</td>
<td>Asn</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Leu</td>
<td>Asn</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Val</td>
<td>Thr</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Leu</td>
<td>Asn</td>
</tr>
</tbody>
</table>

All of the 30 selected Cip-resistant strains studied had at least one mutation in gyrA, most isolates showing Ser83Leu substitution, and 93% had two gyrA QRDR mutations. All isolates harboured at least one QRDR parC substitution, most isolates showing Ser80Ile, and approximately 17% of the isolates had two parC substitutions. In general, isolates with increased numbers of gyrA and parC mutations had higher Cip MICs. qnrB gene was detected in one SHV producing isolate (Cip MIC 16 mg/L, gyrA Ser83Leu, Asp87Asn and parC Ser80Ile). The isolate was positive for qnrB19 by DNA sequencing. qnrB19 and SHV were transferred to E. coli K12 by conjugation. Susceptibility testing confirmed the transfer of resistance to ceftazidime, cefotaxime and aztreonam while the Cip MIC of the transconjugant was as low as <0.03 mg/L. None of 191 ESBL-producing strains harboured qnrA or qnrS.

Conclusions: In general, strains with higher Cip MICs have increased the number of gyrA and parC mutations. The prevalence of qnr genes among our ESBL-producing E. coli isolates is low (0.5%).
The differential effect of mutations in RamR, in mediating antibiotic susceptibility in Klebsiella pneumoniae

J. Findlay, T. Schneider* (Edinburgh, UK)

Objectives: The transcriptional activator RamA confers an antibiotic resistance phenotype in Klebsiella pneumoniae when overexpressed. Recently a tetR-like gene that lies upstream of ramA, known as ramR, has been identified as a repressor of ramA. Correspondingly clinical isolates of K. pneumoniae with mutations within ramR have been shown to overexpress ramA; however ramA overexpression clinical isolates with no changes within the repressor ramR or within the associated promoter regions have also been found. Thus the aims of this study were:

- To firstly determine whether ramA overexpression mediated through ramR-depression was dependent on the selective agent and secondly to determine which mutations within the ramR gene would impact on resistance profiles upon co-selection with wild-type ramR.

Methods: Laboratory mutants from K. pneumoniae Ecil8 were selected for by the culturing of exponential growth phase bacterial cultures on ciprofloxacin or chlorpromazine plates at four times the MIC. The ramR gene was amplified from a selection of the mutants and was sequenced. The subsequent MICs to chloramphenicol (Cm), norfloxacin (Nor) and tetracycline (Tet) of the selected mutants with changes within the ramR gene were then determined. ramR mutants were complemented with a plasmid containing the wild-type ramR, PACramR, and their subsequent MICs were determined to Cm, Nor and Tet as before.

Results: Four of the selected mutants were revealed to harbour mutations resulting in amino acid changes within the ramR gene. The mutations (G96D, S137Stop, E175K) found in the ramR gene appeared to favour the C-terminus region. The mutants exhibited 32–4 fold increases in MICs compared to the parental strain depending on which mutations were sustained within ramR. Complementation with the wild-type ramR resulted in 1–16 fold reductions in the MICs also dependent on the type of ramR mutations.

Conclusion: All the mutants appeared to sustain ramR changes regardless of the compound used in the selection, indicating that ramR is a critical factor in mediating ramA overexpression. The partial restoration of the parental phenotypes in the ramR-mutants indicates the MDR phenotypes are attributable to mutations within the RamR protein but another factor may be required to restore susceptibility to parental levels. The mutations identified within RamR protein are clustered around the C-terminus suggesting the relative importance of this region in the derepression of ramA.

Emergence of AcrAB-mediated tigecycline resistance in a clinical isolate of Enterobacter cloacae

M. Hornsey*, M.J. Ellington, M. Du smith, G. Scott, D.M. Livermore, N. Woodford (London, UK)

Objectives: Tigecycline resistance in the Enterobacteriaceae remains rare in the UK, as elsewhere, but has been associated with up-regulation of the AcrAB efflux system. Using a susceptible and resistant pair of UK clinical isolates, we investigated the role of this pump in the emergence of tigecycline resistance in E. cloacae.

Methods: Two isolates of E. cloacae, identified by API20E, were recovered from a spinal abscess complicating metal work inserted 2 years previously for a fracture of L2 in an adult male. MICs were determined by agar dilution on IsoSensitest agar according to BSAC guidelines. Laboratory mutants were selected from the susceptible isolate in vitro by exposure to increasing concentrations of tigecycline. PFGE was used to determine relatedness. Expression of the acrAB operon was monitored by real-time RT-PCR using primers for acrB and was quantified relative to rpoB. Insertional inactivation of the acrB gene with a gentamicin resistance cassette was mediated by the bacteriophage lambda Red recombination system.

Results: The clinical isolates required tigecycline MICs of 0.5 and 4 mg/L, respectively, whilst the most resistant laboratory-selected mutant required a tigecycline MIC of 32 mg/L. Real-time RT-PCR identified a mean 5× and 12× increase in acrAB transcript in the resistant clinical isolate and final mutant, respectively, compared with the susceptible clinical isolate. Although the PFGE profiles of the wild-type pair were not identical, analysis of the final and intermediate laboratory mutants showed an analogous transition from the PFGE profile of the susceptible clinical isolate to that of the resistant clinical isolate.

Conclusions: Escherichia coli is a problematic nosocomial pathogen, but is generally susceptible to tigecycline. We report the emergence of low-level resistance to this agent in vitro, associated with up-regulation of the AcrAB efflux pump. We also demonstrated in vitro the potential to select highly tigecycline-resistant mutants in this species.

Resistance in Pseudomonas spp.

L. Poiret*, J.D. Decouchet, F. De Luca, A. Verlinde, L. Ide, G.M. Rossolini, P. Nordmann (Le Kremlin-Bicêtre, FR; Siena, IT; Roeselare, BE)

Objectives: Acquired extended-β-lactamases (ESBLs) are rarely identified in Pseudomonas aeruginosa, being of the TEM, SHV, CTX-M, PER,GES,P, and BEL types. In Belgium, the occurrence of BEL-1 producers showing reduced susceptibility to expanded-spectrum cephalosporins has been previously reported. Our study reports the characterisation of a BEL-type variant identified in a P. aeruginosa isolate highly resistant to expanded-spectrum cephalosporins.

Methods: ESBL production was assessed by disk synergy tests. PCR experiments were performed using primers specific for ESBL genes. Plasmid extraction was performed by the Kieser technique. Matting-out assays were performed using P. aeruginosa PU21 as reference strain. Cloning of the PCR amplims was realised in vector pTOPO-Blunt II. Genotyping was performed by using Pulsed-Field Gel Electrophoresis (PFGE), β-lactamase purification was performed by ion-exchange chromatography, and kinetic measurements performed using UV spectrophotometry.

Results: P. aeruginosa isolate 531 was recovered from a urine sample of a patient hospitalised in Belgium in February 2007. It was highly resistant to all β-lactams, except to carbapenems. ESBL test was positive, and PCR revealed the presence of a blaBEL-like gene that was identified as chromosomally-located. Sequencing identified β-lactamase BEL-2 differing from BEL-1 by a single substitution (Leu to Phe at position Ambler 162). Susceptibility testing showed that the E. coli recombinant strain expressing BEL-2 exhibited higher MICs of CAZ, CTX, CRO, and FEP as compared to the isogenic construct expressing BEL-1. Kinetic analysis of purified BEL-2 revealed much lower Km values as compared to BEL-1. PFGE analysis showed that isolate 531 was undistinguishable from the BEL-1-positive isolate 51170 previously reported from Belgium.

Conclusion: This study identified a novel BEL-type enzyme which possesses increased activity against most β-lactams. The Leu162Phe substitution located in the omega-loop of the β-lactamase largely improved apparent substrate affinities, particularly with expanded-spectrum cephalosporins. BEL-2 was identified in a strain that is clonally-related to the original BEL-1 producer and recovered from the same geographical area, although three years later. These data support the hypothesis of the persistence and evolution of a BEL-type ESBL-producing P. aeruginosa clone in Belgium.

BEL-2, an expanded-spectrum β-lactamase with increased activity toward broad-spectrum cephalosporins in Pseudomonas aeruginosa

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**P1486** Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from a French hospital

J.M. Rodriguez-Martinez, L. Poirel*, P. Nordmann (Le Kremlin Bicêtre, FR)

**Objectives:** The aim of the work was to evaluate the contribution of different mechanisms of resistance to carbapenems in *Pseudomonas aeruginosa* including the recently discovered expression of extended-spectrum cephalosporinases (ESACs). Those enzymes correspond to natural AmpCs of *P. aeruginosa* possessing a T105A substitution conferring the ability to hydrolyse carbapenems at low level.

**Methods:** Thirty-two non-repetitive *P. aeruginosa* clinical isolates recovered in 2007 being resistant or of intermediate susceptibility to imipenem (IMP) were studied. MICs were determined by agar dilution and E-test techniques. The level of expression of the AmpC β-lactamases was determined by UV spectrophotometry. PCR and sequencing were used to characterise the blampC genes. Overexpression of AmpC was evaluated by using Mueller-Hinton agar plates containing cloxacillin (AmpC inhibitor). PCR, sequencing and SDS-PAGE were used to characterise the outer-membrane protein OprD. The level of expression of OprD, and of the efflux systems MexAB-OprM, MexXY-OprM and MexCD-OprJ were determined by real time RT-PCR assays.

**Results:** 87% (*n* = 28) of the isolates were resistant to IMP. The main mechanism involved in that resistance was loss of OprD. 78% of the isolates contained modifications leading to the inactivation of OprD, and a reduced expression of OprD compared to PAO1 was noticed in the remaining isolates (Table).

**Conclusions:** Resistance to imipenem and meropenem in *P. aeruginosa* is multifactorial, combining loss of OprD porin, overexpression of ESACs and efflux pumps overexpression.

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**P1487** Molecular epidemiology and β-lactamase resistance mechanisms of ceftazidime-resistant *Pseudomonas aeruginosa* causing blood infections in a Brazilian hospital

R. Pica, L. Poirel*, A. Gales, P. Nordmann (Le Kremlin Bicêtre, FR, Sao Paulo, BR)

**Objective:** To investigate the mechanisms responsible for β-lactam resistance among a collection of ceftazidime-resistant *P. aeruginosa* recovered from blood samples in a University Hospital in São Paulo, Brazil, during a one-year period. The spread of extended-spectrum β-lactamases (ESBL) or metallo-β-lactamase (MBL) was evaluated.

**Methods:** 154 non-repetitive *P. aeruginosa* were recovered from blood cultures of patients hospitalised in São Paulo in 2005. 43 isolates (28%) displayed ceftazidime resistance and were further studied. Susceptibility testing and ESBL detection was performed by disk diffusion and synergy tests. Molecular typing was performed by PFGE. PCR and sequencing was used to identify β-lactamase genes and their genetic environment. Studies of the genetic support of β-lactamase-encoding genes were performed by plasmid hybridisation, transformation experiment and the I-Ceu1 technique.

**Results:** Resistance rates for the 43 ceftazidime resistant isolates were over 80% for carbapenems, aminoglycosides and quinolones.

**Conclusions:** This study underlines the spread of MBL-producing *P. aeruginosa* as well as the emergence ESBL production in that species, including ESBL with carbapenemase property (GES-5).
**P1489** Study of metallo-$eta$-lactamase production in clinical isolates of pan-resistant *Pseudomonas aeruginosa*

L. Fung, I. van der Heijden, A.K. Mostacho, A.S. Levin, I. Bozzoncini, F. Rossi, J. Paez, S.F. Costa*
(Sao Paulo, BR)

Metallo-$eta$-lactamases (MBLs) are being reported with increasing frequency and from several countries worldwide. Several phenotypic tests have been developed for MBL detection, such as the MBL Etest, double-disc synergy tests (DDST), combined disk assay, and microdilution test. All of these tests are based upon the ability of chelating agents, EDTA and thiol-based compounds, to inhibit the MBL activity. However, an international consensus on appropriate phenotypic test to detect MBL is lacking.

**Objective:** Compare MBL phenotypic production and detection of MBL genes by multiplex PCR in pan-resistant (susceptible to polymyxins) clinical isolates of *P. aeruginosa*.

**Material:** 104 *P. aeruginosa* isolates pan-resistant from 02 Brazilian hospitals were screened for MBL production by DDST assay employing carbapenem and ceftazidime disk to which EDTA and 2-mercaptopyrrolionic acid (MPA) were added, minimum inhibitory concentration (MIC) reduction test with phenanthroline and Etest containing Imipenem and EDTA. Disk Diffusion susceptibility to Aztreonam and MIC microdilution of $eta$-lactams, quinolones and aminoglycosides were done according with the CLSI. Multiplex PCR using specific primers for IMP, VIM, SPM, GIM and SIM was performed to confirm the presence of the MBL genes. PFGE was done to evaluate the clonality of isolates.

**Results:** MIC50 and MIC90 for Imipenem and Meropenem were respectively 64 g/ml, 256 g/ml, 32 g/ml and 256 g/ml. MBLs were detected by multiplex PCR only in 18 isolates (17%). SPM was the most frequent MBL being present in 14 isolates, followed by VIM in 3 and IMP in 1 isolate. PFGE showed that 50% of SPM positive strains belong to a predominant clone. Two MBL positive isolates were resistant to Aztreonam, DDST using Cefazidime detected production of MBL in 3 strains, and using Imipenem disk only in 1 strain. MIC reduction test was positive in 55% of MBL positive strains. MBL Etest was positive in all PCR MBL positive isolates, with sensitivity of 100%, specificity of 16% and positive predictive value (PPV) of 4%. The combination of MIC reduction test and MBL Etest showed specificity of 98%, sensitivity of 55%, PPV of 90% and predictive negative value of 86%.

**Conclusions:** MBL presence was less frequent than expect. MBL Etest was the most sensitive phenotypic method to detect MBL, however showed low specificity. The combination of MIC reduction test and MBL Etest increased the specificity of phenotypic detection of MBL.

**P1490** Molecular epidemiology of metallo-$eta$-lactamase-producing *Pseudomonas putida* in a Spanish hospital

C. Juan*, L. Zamorano, S. Alberti, J.L. Pérez, A. Oliver (Palma, ES)

**Objective:** To study the prevalence, nature, involved genetic elements, and the molecular epidemiology of metallo-$eta$-lactamase (MBL)-producing *P. putida* strains isolated in a Spanish hospital between 2005 and 2008.

**Methods:** Etest and API 20NE were used, respectively, for the susceptibility testing and identification of *P. putida* clinical isolates. The MBL Etest was used for screening, and was followed by the amplification of blaVIM genes by PCR. The clonal relatedness between the isolates was evaluated by pulsed-field-gel-electrophoresis (PFGE). The plasmids harbouring the MBL genes were characterised and compared through the analysis of the restriction (BamHI-HindIII) fragments length polymorphisms (RFLP) followed by Southern blot hybridisation using blaVIM probes. Additionally, electroporation of the plasmids to *P. aeruginosa* PA01 was attempted. The genetic composition of the integrons harbouring the MBLs was investigated by PCR and sequencing, following previously described protocols.

**Results:** MBL-producing *P. putida* was detected in clinical samples (1 urine, 1 sputum, 3 blood, 1 vascular catheter, and 2 peritoneal fluid) from 8 patients, representing 14% of all the infections by *P. putida* group strains during the study period. In contrast, MBL production was detected in only 0.32% of *P. aeruginosa* infections during the same period. PFGE revealed that the 8 *P. putida* isolates belonged to 8 different clones, 2 of them harbouring blaVIM-1 and 6 blaVIM-2. All the strains showed resistance or reduced susceptibility to gentamicin and tobramycin, and half of them were additionally resistant to ciprofloxacin. Southern blot revealed that all the MBLs, except 1 VIM-2, were plasmid-located. An important plasmid diversity was also denoted, since RFLP analysis yielded 6 different patterns among the 7 blaVIM-encoding plasmids. On the other hand, all the MBLs were found to be encoded in class 1 integrons, that showed conserved structures among the different isolates for each MBL: intI1-sacA4-blaVIM-2 and intI1-blaVIM-1-sacA4-aadA1, respectively.

**Conclusion:** The alarmingly high proportion and clonal diversity of MBL-producing *P. putida* clinical isolates suggest an important environmental reservoir of these highly relevant resistance determinants. Therefore, considering the threat of potential horizontal transfer of these plasmid-located MBL-encoding integrons to other species such as *P. aeruginosa*, active surveillance is warranted.

**P1491** Ability of Spanish clinical laboratories in detecting MBL-producing strains of *Pseudomonas*: results from the SEIMC QC programme


**Background:** Although rare, MBL-producing strains of *Pseudomonas* are increasingly reported in Spain. Interpretation of resistance pattern to $eta$-lactams could be difficult when several mechanisms coexist in a strain. Genetic determinants for MBL are located in transposons coding for resistance to other drugs. Then, it is crucial that clinical laboratories were able to recognize this infrequent, but dangerous, mechanism. The Spanish Society of Clin Microbiol & Infect Dis (SEIMC) has launched a QC Programme, being continuous education one of its most distinctive features. We present results of a recent QC with a MBL-producing *P. putida* strain.

**Methods:** A VIM-1 MBL-producing strains was sent to 281 participants along with a brief clinical history (pneumonia in an oncology patient not responding to imipenem). Participants were asked to identify the strain, perform susceptibility tests, and make relevant comments related to the case. Identification of a specific resistance mechanism was not asked. The strain was characterised by a reference laboratory that report resistance to all $eta$-lactams, including cefepime, aztreonam and carbapenems, as well as gentamicin and tobramycin, remaining susceptible only to amikacin and colistin. VIM-1-type MBL was determined by sequencing.

**Results:** The 88.2% of participants answered this QC. Results of the susceptibility studies to $eta$-lactams were almost coincident with that of the reference laboratory, including resistance to carbapenems. Only 67.8% agreement was observed for tobramycin (9% major discrepancies). A total of 29 (11.7%) reported the presence of a MBL, and 11 additional participants suggested the possibility. Some of these also remark the possible existence of an additional mechanism explaining this phenotype (resistance to aztreonam). Four participants reported presence of several resistance determinants (no particular one detected). Although there was a place in the form for reporting “special features”, 167 (67.5%) laboratories gave no information about it.

**Conclusion:** Only a modest percentage of participants detected or suspected the MBL (16.1%). Most of these correctly reported the susceptibility phenotype. Implementing tests for MBL detection (impenem-EDTA) is a need for the future. According with the experience of the SEIMC QC Programme with other resistance determinants (e.g. ESBL-producing enterobacteria), a notorious improvement is expected when the strain were sent again in the future.
Classical epidemiology of the disease stands for one of the most important pathogens in nosocomial setting. Understanding the pathogen distribution and relatedness is essential for determining the epidemiology of hospital infections and aiding in the design of rational pathogen control methods. In this work we proposed to characterise PA VIM-2 producers belonging to two central hospitals of Portugal by random amplification of polymorphic DNA (RAPD) to understand if isolates are intra or intra related between hospitals.

**Methods:** Of the 27 VIM-2 producing isolates, 15 were from Hospitals da Universidade de Coimbra (HUC) collected in first semester of 2008 and were from Neurotraumatology (WNT), Surgery III (WS), Hepatic Transplant Unit (HTU), Cardiology (WC), Medicine (WMI), Orthopedy (WO), Infectious (W), and Emergency (ER). Twelve were collected from Centro Hospitalar de Coimbra (CHC) during a year (2007–2008), and were from wards of Pneumology (WP), Neurosurgery (WN), Infectious (WI), Medicine (WM), Paediatric Hospital (PH), and Hospital of Pombal (HP). The samples were from different products, namely, urine, sputum, blood, and exudates. The minimal inhibitory concentration (MIC) of the MICs of the VIM-2 strains was determined by E-test method. DNA was amplified using the primer 5′-AGCGGGCCAA-3′. We assigned a letter for each different RAPD profile.

**Results:** Among the 27 blaVIM-2 isolates the MICs revealed that aztreonam inhibited 81.5%, followed by piperacillin 66.7%, ceftazidime 25.9%, and meropenem 22.2%. RAPD typing generated 14 different patterns (A to O). Seven patterns were from HUC (A to G) and the most prevalent was pattern A that appeared to be disseminated in 3 wards namely WNT, WS and HTU (seven strains), and other patterns were represented by two or one strain. The patterns H to O belonged to CHC where H pattern was predominant (five strains) and appeared in different wards of PH, other profiles were constituted by one or two strains. Identical RAPD patterns between the two hospitals were not seen.

**Conclusions:** Fourteen genotypically different strains were identified, indicating that prevalence of carbapenemases-encoding genes was mainly due to a gene spread and in a lesser extent to clonal dissemination. The possibility of spreading of VIM-2 in Gram negative pathogens could emerge as a great problem in the clinical setting and underscores the need for systematic surveillance of these resistant determinants.

**Detection of VIM-2 metallo-β-lactamase in Pseudomonas aeruginosa isolates from two central hospitals in Portugal**

T. Reis*, G. Ribeiro, C. Vital, A. Alves, O. Cardoso (Coimbra, PT)

**Objective:** Pseudomonas aeruginosa (PA) is one of the leading causes of nosocomial infections as it is an opportunistic human pathogen with an amazing capacity to resist antibiotics either intrinsically or following acquisition of resistance genes. The aim of this study was to identify metallo-β-lactamases (MBLs) in imipenem resistant isolates obtained from two central hospitals belonging to the centre region of Portugal.

**Methods:** Imipenem resistant bacterial isolates (n = 91) from Centro Hospitalar de Coimbra (CHC) were collected from June 2007 to June 2008 (one year) and 31 from Hospitais da Universidade de Coimbra (HUC) collected from January 2008 to June 2008 (half year). Double disk synergy test was used for screening MBLs. For research of blaVIM, blaIMP, blaGIM, and blaSPM, PCR was done. PCR products obtained were sequenced and analysed. The minimal inhibitory concentration (MICs) of the VIM-2 isolates was determined by E-test method.

**Results:** The double disk synergy test was positive in 32 isolates from CHC and in 18 of HUC. The presence of blaVIM was positive in 12 from CHC and 15 from HUC, and DNA sequencing showed the presence of blaVIM-2 gene in all of the 27 isolates (54%). The other metallo-β-lactamases tested were not observed. Among the strains that harboured VIM-2, MICs were determined and the results revealed that aztreonam inhibited 81.5% followed by piperacillin 66.7%, ceftazidime 25.9%, and meropenem 22.2%.

**Conclusions:** Our findings are of concern since they demonstrate that VIM-2 can emerge and turn into a major cause of broad spectrum β-lactam resistance among nosocomial pathogens. The monitoring of these non-fermenting Gram negative bacilli for production of metallo-β-lactamases should become a standard aspect of any local or global surveillance systems.
Multiresistant epidemic clones of *Pseudomonas aeruginosa* in the Czech Republic

A. Nemec*, M. Maksimenko, L. Krizova, M. Musilek (Prague, CZ)

**Objective:** To determine whether the high prevalence of antimicrobial resistance among bloodstream isolates of *Pseudomonas aeruginosa* in the Czech Republic is associated with the clonal spread of multidrug resistant (MDR) strains.

**Methods:** The study included 108 bloodstream isolates, which were selected from 437 isolates of *P. aeruginosa* collected in the Czech Republic within the European Antimicrobial Resistance Surveillance System (EARRSS) project in 2007. The 108 isolates originated from 49 hospitals in 36 cities. They were tested for susceptibility to piperacillin, cefazidime, ceftazidime, meropenem, imipenem, ciprofloxacin, gentamicin, tobramycin, amikacin and colistin by E-test. The genotypes of the isolates were assessed by multilocus sequence typing (MLST), macrorestriction analysis of genomic DNA and class 1 integron typing.

**Results:** Forty-six isolates were susceptible to all antimicrobial agents when 16 and 46 isolates were resistant or intermediate to 1–3 and 4–9 agents, respectively. A total of 41 multilocus sequence types (ST) were identified, which, except for four unique STs, differed from each other in at least three allelic states. ST235 and ST175 encompassed 34 (74%) of 46 isolates resistant to more than 3 agents. Class 1 integrons were found in 47 MDR isolates, with at least 18 different integron variable regions. Twelve isolates with ST235 harboured an integron with a 1.9 kb variable region while 15 isolates with ST175 shared an integron with a 1.6 kb variable region.

**Conclusion:** The high prevalence of antimicrobial resistance in *P. aeruginosa* isolates in the Czech Republic is predominantly associated with two MDR epidemic clones, one of which (ST235) belongs to international clonal complex CC11. Supported by grant NR/9428–3 of the Ministry of Health of the Czech Republic.

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Emergence and persistence of multidrug-resistant *Pseudomonas aeruginosa* serogroups O11 and O12


**Objectives:** In 1989 Pitt et al. (Epidemiol Infect. Dec;103(3):565–76) reported the emergence of a European multidrug resistant (MDR) serotype O12 clone, and in 1998 Tassios et al. (J Clin Microbiol. Apr;36(4):897–901) reported the emergence of MDR in the ubiquitous and dominant serogroup O11. The objective of the present study was to investigate the emergence, spread and actual status of these *MDR P. aeruginosa* serogroups in the light of the global population structure.

**Methods:** 328 unrelated *P. aeruginosa* clinical strains were checked for serogroup and serotype. The prevalence of 23 *Antibiotic Resistance Genes* (ARGs) (15 coding for β-lactamases and 8 for aminoglycoside-modifying enzymes) was determined by PCR. The MIC values for 21 antibiotics were determined using the VITEK 2 Advanced Expert System (AES).

**Results:** In the minimum spanning tree, based on the combination of the characteristics from the 328 strains, we identified 11 clonal complexes (CCs). Fifty-nine strains (22.4%) were MDR, including 14 O11 (23.7%) and 17 O12 (28.8%) strains. Forty-eight of the 58 detected ARGs were found in MDR O11 and O12 strains. Twenty MDR O12 strains, isolated in 9 countries, some of them separated by thousands of miles, were shown to cluster into a very conserved clone. Only clinical non-CF strains isolated post 1980 clustered into this clone. The MDR serotype O11 strains showed, with the exception of some clonal strains, an overall higher genetic divergence. They belonged to 2 distant CCs, which also included environmental and animal strains, but not CF strains. Most members of the O12 clone harboured the two original (1989) ARGs (PSE-1 and AAC(6’)-II), while others harboured recent ARGs (e.g. VIM-8). The prevalence of 23 ARGs was 8 for aminoglycoside-modifying enzymes and was determined by PCR. The MIC values for 21 antibiotics were determined using the VITEK 2 Advanced Expert System (AES).

**Conclusion:** We suggest that MDR O11 *P. aeruginosa* epidemic strains are members of two widespread and successful CCs that were selected from the environment, in different locations and on several occasions, adapted to the high care niche, and dispersed in hospitals. MDR O12 strains are probably the offspring of a minority clone, which was locally
selected from the environment, adapted to the high care niche, and rapidly spread across high care facilities all over the world.

**P1499** Correlation between antibiotic resistance gene detection and antibiotic resistance phenotype in *Pseudomonas aeruginosa* strains


**Objectives:** *P. aeruginosa* exhibits high inherent antibiotic resistance (ABR) combined with acquired ABR mechanisms. Important acquired ABR mechanisms in *P. aeruginosa* are β-lactamases (BL) and aminoglycoside-modifying enzymes (AME). The objective of this study was to analyze the relation between the presence of ‘Antibiotic Resistance Genes’ (ARGs) and the ABR phenotype.

**Methods:** 328 unrelated *P. aeruginosa* clinical CF (43) and non-CF (142), animal (63) and environmental (55) strains, collected between 1882 and 2008 in 69 localities (30 countries, 5 continents) and characterised by, amongst others, FAFLP fingerprinting, serotyping and MLST were screened, by PCR, for the presence of 23 ARGs (15 coding for BLs and 8 for AMEs). The MIC values for 21 antibiotics were determined using the VITEK 2 Advanced Expert System (AES). An arbitrary ABR index (the area of the region bounded by the graph and the x-axis in figure 1) was determined. Additionally, the oprD gene was screened for ‘defective oprD mutations’ (DOMs) that confer carbapenem resistance.

**Results:** Fifty-eight ARGs were detected. Most of them (48) were found in multidrug resistant (MDR) epidemic strains exhibiting serotypes O11 and O12. None of the 43 CF strains exhibited ARGs. We observed a gradient in ABR phenotype (ABR index), from strains isolated in the pre-antibiotic era (0.63) to the contemporary serotype O12 epidemic strains (3.63), over environmental (1) and animal (1.31) strains. There was a total absence of MDR among the 49 strains isolated before 1980. The ABR index of the CF isolates (2.05) was comparable to that of clinical non-CF isolates (2.25), but showed a broader distribution (Figure 1). This is probably the result of differences in ABR mechanisms. Inherent ABR mechanisms like efflux pumps and biofilms cause broad spectrum ABR and are more common in CF isolates. Most of the clinical non-CF strains showed either a moderate or a very high ABR, depending on whether they acquired ARGs or not. Twenty-one distinct DOMs mediated resistance to MER in 22 clinical strains, including 7 CF strains. Overall, there was a satisfactory correlation between the detected ARGs and the ABR phenotype.

**Conclusion:** Since the introduction of antibiotics in clinical practice ABR is spreading, also in the environment, and has reached dramatic levels in some MDR *P. aeruginosa* clones. The detection of ARGs through PCR has potential to generate partial, but rapid, information regarding ABR in non-CF *P. aeruginosa* strains.

**P1500** Resistance to carbapenems and ciprofloxacin of intensive care unit *Pseudomonas aeruginosa* isolates in relationship to the ICU antimicrobials use at a university hospital, Zagreb, Croatia

V. Erdeljić*, I. Francetic, S. Kalenic, Z. Bosnjak, A. Budimir, K. Makar Ausperger, R. Likić, L. Bielen, M. Radić Acumiler (Zagreb, HR)

**Objectives:** The selection of resistant bacteria as a result of wide use of antibiotics is predictable and cannot be avoided. However, non-adherence to other infection control measures is an often underestimated problem that deserves attention. Despite the lack of a surveillance system, the problem of multi-drug resistant *P. aeruginosa* strains has been well established in our hospital, especially in the Intensive Care Units (ICUs). We have evaluated trends in *P. aeruginosa* resistance to carbapenems and ciprofloxacin in the ICU of the Department of Medicine during a period of 9 months with regard to antibiotic consumption.

**Methods:** Data on antibiotic use were collected from the hospital's pharmacy. The numerator used was DDD (defined daily dose); the denominator used was per-100 admissions. Data on admissions were collected from the administration office. Microbiology data were obtained from the Department of Clinical Microbiology. The evaluation period was: November 2006–July 2007.

**Results:** During the evaluated period, the hospital's microbiology laboratory isolated 61 non-duplicate *P. aeruginosa* strains. The average observed monthly percentages of ciprofloxacin, piperacillin/tazobactam, imipenem/cilastatin and meropenem-resistant *P. aeruginosa* were 55.8% (0–100%), 32.9% (0–83.3%), 51.9% (0–91.7%) and 44.5% (0–91.7%) respectively. The observed results are higher than average hospital resistance rates of *P. aeruginosa* to these antibiotics (36%, 22%, 32% and 30% respectively). Out of 32 antibiotics, the most prescribed in the evaluated ICU were amoxicillin/clavulanic acid (14.8%), ciprofloxacin (9.4%), carbapenems (9.3%), azithromycin (7.4%) and piperacillin/tazobactam (6.9%). Correlating the monthly resistance rates with antibiotic consumption did not demonstrate significant relationship between carbapenems, piperacillin/tazobactam, imipenem/cilastatin and meropenem consumption and *P. aeruginosa* resistance rates to these antibiotics.

**Conclusion:** The results show disturbingly high resistance of *P. aeruginosa* to carbapenems and ciprofloxacin in the evaluated ICU, without causative relationship to antibiotic usage. This indirectly points out towards non-compliance with infection control measures and to bacterial spread of resistant strains of *P. aeruginosa* in the evaluated ICU. Surveillance systems aimed at monitoring not only antibiotic use, but also other infection control measures are lacking and should be implemented as soon as possible.

**P1501** *Pseudomonas aeruginosa* clones disseminated among patients in a tertiary care teaching hospital in Greece during a two-year period

M. Koutsiogiannou*, E. Drougka, E.D. Anastasiou, M. Christofidou, I. Spiliopoulou (Patras, GR)

**Objective:** *Pseudomonas aeruginosa* is a cause of a wide diversity of infections in immunocompromised hosts. The high level of antibiotic resistance combined with the frequent spread of epidemic strains make *P. aeruginosa* one of the major nosocomial pathogens. Antibiotic resistance patterns, serotypes and clones were determined in *P. aeruginosa* isolates recovered from clinical samples of different hospitalised patients during a two-year period.

**Methods:** A total of 220 *P. aeruginosa* isolates recovered from inpatients during 2006–2007 were identified at species level by standard methods (Oxiferm, BD, BBL). Antibiotic susceptibility testing was performed by the agar disk diffusion method according to CLSI guidelines. MIC of colistin (CL) was determined by the Etest (AB Biodisk) and the production of metallo-β-lactamases (MBL) was tested by the double strip Etest. Serotyping was performed by 17 monovalent antisera against
the O antigen according to the International Antigenic Typing Scheme. Clones were defined by PFGE of chromosomal DNA Spel digests.

Results: Eighty-four isolates were recovered from patients hospitalised in the Intensive Care Unit (ICU), followed by the Departments of Internal Medicine (74), Surgery (23), Paediatrics (20) and Outpatients (19). Sixty-one P. aeruginosa were isolated from respiratory tract samples from the ICU, followed by wounds’ infections (56), bacteraemias (37), urinary tract (37), catheters (12) and stool specimens (17). Multi-resistant isolates were 52% and 61% in 2006 and 2007 respectively; MBL-positive isolates were 42% (19), while no isolate was resistant to colistin. The predominant serotype was O:11 (112 isolates), followed by O:1 (18). Eighty clones were identified by PFGE, with five predominant: A (74 strains), B (9 strains), C (6 strains), D (28 strains) and S (5 strains). Four out of five clone S strains were recovered from children. These clones were dominant in the hospital during the two-year period. Serotype O:11 strains were classified into clones A and D, isolated mainly from the ICU. Among MBL-positive strains 16 belonged to clone D and were serotype O:11, six to clone A and four to clone B.

Conclusions: Multi-resistant P. aeruginosa strains are disseminated in our hospital, mainly among ICU patients. Polyclonality combined with the spread of certain dominant clones including multi-resistant strains, indicate the need of appropriate antibiotic policy and continuous infection control measures.

**P1502** Prevalence and epidemiology of antibiotic resistance in _Pseudomonas aeruginosa_ isolated from low respiratory tract of patients hospitalised in intensive care units from 5 Belgian hospitals, 2004–2008


**Objectives:** _Pseudomonas aeruginosa_ (PA) is a major cause of nosocomial infections, with one of its preferential “niches” in respiratory tract of patients in ICU. Our objective was to evaluate the level of resistance of PA towards commonly used antibiotics in this setting.

**Methods:** 138 first, non-duplicate isolates were collected from 5 hospitals over the last 4 years from ICU patients with a suspicion of nosocomial pneumonia (confirmed in most cases by retrospective analysis of medical records). MICs of 5 commonly used antibiotics plus ticarcillin and aztreonam (as efflux reporters) were determined by geometric microdilution in cation-adjusted Muller-Hinton broth. Susceptibility was assessed according to EUCAST Breakpoints (BP).

**Results:** Based on EUCAST breakpoints, and using a 20% resistance cutoff, only amikacin could be considered effective globally as well as in each individual hospital. Meropenem was globally effective, but resistance exceeded the cut-off in 3/5 hospitals. Gentamicin, aztreonam, ciprofloxacin and cefepime were globally ineffective, with resistance exceeding 40% for cefepime in 2 hospitals (cefpime-resistant isolates were also often resistant to other antibiotics [GEN, 24%; AMK, 8%; ATM, 27%; MEM, 20%; and CIP, 20%]).

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<th>MEM</th>
<th>CIP</th>
<th>TET</th>
<th>FEP</th>
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</thead>
<tbody>
<tr>
<td>MIC50</td>
<td>4/32</td>
<td>0.0/30</td>
<td>0.0/30</td>
<td>6.0/32</td>
<td>67/33</td>
<td>16/32</td>
<td>12/64</td>
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<tr>
<td>MIC90</td>
<td>4/32</td>
<td>0.0/30</td>
<td>0.0/30</td>
<td>67/33</td>
<td>16/33</td>
<td>16/32</td>
<td>64/64</td>
</tr>
</tbody>
</table>

**Conclusion:** The level of antibiotic resistance in _Pseudomonas aeruginosa_ (including cross-resistance, as illustrated for cefepime) in the ICU surveyed is critically limiting therapeutic options, but in variable way that justifies early and careful assessment of susceptibilities for ensuring appropriate therapy. Efflux-mediated resistance seems also very prevalent and will need appropriate diagnostic approaches.

**P1503** _Pseudomonas aeruginosa_ and the hospital: antimicrobial susceptibility trends over a four-year observation period

R. Manfreli*, A. Nanetti (Bologna, IT)

**Introduction:** A prospective microbiological surveillance monitoring including culture and systematic in vitro antimicrobial susceptibility studies of all relevant pathogens, is ongoing at our Hospital. Particular attention has been deserved to _Pseudomonas aeruginosa_, and a leading Gram-negative, often multiresistant organisms in hospital settings.

**Materials-Methods:** The temporal variations of in vitro antimicrobial sensitivity rates of all isolated _Pseudomonas aeruginosa_ strains were collected for all suitable isolates, during the four-year period ranging from January 2004, up to December 2007. The same pathogen cultured more than once from the same patient within one month, was considered one time only (one episode).

**Results:** Among _Pseudomonas aeruginosa_ isolates (2,083 evaluated tested strains), the best performance was obtained by the old colistin (colimycin), with a sustained 100% susceptibility rate, followed by amikacin (72.8–81.2% of tested strains), imipenem (76.8–80.8%), piperacillin-tazobactam (70.9–78.7%), cefazidine (68.9–77.1%), and tobramycin (64.6–70.9%). On the other hand, gentamicin (53.1–63.5% of tested strains), aztreonam (57.5–66.8%), ciprofloxacin (57.5–65.0%), ticarcillin-clavulanate (53.7–60.5%), and mezlocillin (48.4–55.2%), proved less affordable. A significant temporal trends towards a reduced antibiotic sensitivity was found for the majority of tested molecules, but it resulted significant for aztreonam, ciprofloxacin (p < 0.001), ticarcillin-clavulanate (p < 0.02), and mezlocillin and tobramycin (p < 0.04).

**Conclusions:** A prospective monitoring of antimicrobial susceptibility rates of a major hospital-associated organism like _Pseudomonas aeruginosa_ is relevant, to add to local and national guidelines of antibiotic treatment and prophylaxis. Despite a significant increase of resistance rates against the majority of compounds which usually test active against _Pseudomonas aeruginosa_, however amikacin, carbapenems, piperacillin-tazobactam, amikacin, imipenem, and cefazidime still maintain a reliable role in eventual, empiric regimens to be added pending microbial isolation and in vitro sensitivity assays, since they remained active in at least 70% of hospital isolates of the last four years (2004–2007). Colistin, which maintains full in vitro activity against all _Pseudomonas aeruginosa_ strains, remain as a possible component of combined antimicrobial strategies, when multiresistant pathogens are of concern.

**P1504** Incidence of _Pseudomonas aeruginosa_ resistant to colistin in a tertiary care hospital

E. Garduño*, M. Fajardo, R. Hidalgo, M. Sánchez-González, J. Rangel, J. Blanco-Palennciano (Badajoz, ES)

**Objective:** Infections caused by multidrug-resistant _Pseudomonas aeruginosa_ pose a serious problem due to the limited number of antimicrobials available for treatment, which sometimes requires the use of old antibiotics, such as colistin. The widespread use of this antibiotic in our hospital due to an outbreak of multidrug-resistant _Acinetobacter baumannii_ has led to the appearance of strains of _P. aeruginosa_ resistant to colistin (Pae-CR). The aim of this study is to analyze the incidence of Pae-CR and describe its clinical and epidemiological characteristics.

**Methods:** A retrospective study of patients with Pae-CR isolates during the years 2006–2008 was conducted. The sensitivity to colistin was determined by the MicroScan Walkaway automated system (Siemens Healthcare), and resistance was confirmed using the Etest method (Izasa). We related the annual consumption of colistin in our hospital to the proportion of Pae-CR to that same year.

**Results:** 16 patients with one or more isolates of Pae-CR were retrieved: 6 patients in 2006, 8 in 2007 and 2 in 2008. The percentage of Pae-CR for
Resistance in Pseudomonas spp.

total P aeruginosa was 6.5% in 2006, 4.34% in 2007 and 3.84% in 2008. Colistin consumption in our centre was 12,796 units in 2006, 6041 in 2007 and 160 in 2008. Analysing data by the chi-square method we found no statistically significant differences with respect to the consumption of colistin with the number of isolations of Pae-CR between 2006 and 2007, but there is a statistically significant relationship between the years 2007–2008 and 2006–2008 (p = 0.0139 and p = 0.03796, respectively). The origin of the samples was: 6 respiratory samples, 6 wound exudates, 2 catheter tips, 1 blood and 1 skin. All the isolates, except the skin, were considered causing infection. The age range of patients was 41–72 years (median = 50); 62.5% were men; 50% had been or were in ICU. 4 patients were conformed with A. baumannii, 2 with extended-spectrum β-lactamase producing Escherichia coli and 2 with methicillin-resistant Staphylococcus aureus. Only the four patients conformed with A. baumannii were treated with colistin.

Conclusions: In our hospital the incidence of Pae-CR is low, with a tendency to decrease directly related to the decline in the use of colistin. The emergence of resistance seems not to be associated with exposure to colistin of the isolates of P aeruginosa studied.

**P1506**

**Resistance and epidemiology of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients: a French multi-centre study**

A. Mérens*, C. Llanes, C. Pourcel, M. Roussel-Delvallez, G. Vergnaud, J.D. Cavallo, P. Pliéziat on behalf of the GERPA Study Group

**Objectives:** To describe the epidemiology and the resistance of *P aeruginosa* strains isolated from cystic fibrosis (CF) patients in France.

**Methods:** 204 *P aeruginosa* strains isolated from 153 CF patients (from 1 to 45 years old) were collected in 10 French University-affiliated hospitals in 2007. Their susceptibility (MICs) to 14 antipseudomonal antibiotics was determined by the standard broth microdilution method. Resistance mechanisms to β-lactams were assessed phenotypically (susceptibility profiles in the presence/absence of clavulanic acid or EDTA), by isoelectrofocusing, PCR, and gene sequencing. The enzymatic activities of chromosomally-encoded β-lactamase AmpC were determined spectrophotometrically. The expression levels of efflux pumps MexAB-OprM and MexXY-OprM were quantified by real-time RT-PCR. The expression of mexEF-oprM, mexCD- oprJ, mexEF-oprN and mexXY-oprM efflux, ampC [β-lactamase and oprD porin genes was analyzed by real-time RT-PCR and compared with strain PA01. One gene was assayed from each efflux system. Carbapenemase activity was assessed by modified Hodge and EDTA synergy tests, with carbapenemase genes sought by PCR.

**Results:** 25 *P aeruginosa* isolates were studied, collected from UK CF patients in 2006–8; 7 *P aeruginosa* strains with characterised efflux and AmpC served as controls. MICs were determined by agar dilution and interpreted vs. EUCAST guidelines. Expression of mexAB-oprM, mexCD-oprJ, mexEF-oprN and mexXY-oprM efflux, ampC [β-lactamase and oprD porin genes was analyzed by real-time RT-PCR and compared with strain PA01. One gene was assayed from each efflux system. Carbapenemase activity was assessed by modified Hodge and EDTA synergy tests, with carbapenemase genes sought by PCR. Prescription variations were used as variables in a Student’s t test to evaluate associations with antibiotic resistance; those significantly associated with a resistance (P < 0.05) were used as covariates in a logistic regression model.

**Conclusions:** RT-PCR proved useful for investigating the multiplicity of mechanisms present in these complex *P aeruginosa* isolates from CF.

**P1507**

**β-lactamate evolution activity of *Aeromonas hydrophila* CPha metallo-β-lactamate by design of a chimeric CPha-VIM-1 enzyme**

M. Perilli*, C. Di Listo, S. Rainaldi, G. Celenza, M. Galleni, P.S. Mercuri, C. Pellegrini, C. Forcella, P. Bellio, G. Amicosante (L’Aquila, IT; Liége, BE)

**Objectives:** Metallo-β-lactamases are zinc enzymes belonging to molecular class B. They are able to hydrolyze β-lactam antibiotics in particular carbapenems. Among subclass B2 β-lactamases, *Aeromonas hydrophila* CPha enzyme efficiently hydrolyses only carbapenems while VIM-1, belonging to subclass B1, hydrolyses a broad array of β-lactam antibiotics including penicillins and cephalosporins. CPha (254 aa, 25 kDa, pI 8.0) and VIM-1 (266 aa, 20 kDa, pI 5.2) contain α-β-α-β sandwich structure with one and two zinc ions, respectively, essential to the hydrolysis reaction. The goal of this study was to design and produce a new chimeric enzyme from CPha and VIM-1 in order to improve the catalytic efficiency of CPha against non-carbapenem β-lactam antibiotics.

**Methods:** The construction of chimeric enzyme was performed by overlapping three different DNA segments obtained from PCR amplification of blaCPha and blaVIM-1 genes. Automatic DNA sequencing was performed on PCR fragments and recombinant plasmid using an automatic sequencer ABI-PRISM 310. blaCPha-VIM-1 gene was generated by a PCR-overlap using blaCPha and blaVIM-1 genes.
Prevalence of class 1 and class 2 integrons among Escherichia coli isolates of human and animal origin in Lithuania

J. Pavalionis*, V. Seputienė, M. Ruzauskas, R. Sjugzdžiene, A. Pavilonis, E. Suziedeliene (Vilnius, Kaisiadorys, Kaunas, LT)

Objectives: Investigation of the prevalence and diversity of class 1 and class 2 integrons in 232 Escherichia coli isolates of human and animal origin obtained during period 2005–2008 in Lithuania.

Methods: Total 232 isolates of E. coli from various clinical specimens collected in 5 hospitals in Lithuania (n=98) and from various disease condition and healthy animals (n=134, poultry, swine, cattle) were tested for their antimicrobial susceptibility by disk diffusion method. Isolates resistant to at least two antimicrobials were selected for further studies. PCR and RFLP based analysis were used for the detection of class 1 and class 2 integrons. Resistance gene cassette structure was determined by DNA sequencing of variable parts of integrons. Plasmid location of integrons was confirmed by the conjugation experiments.

Results: One hundred out of 232 (43%) E. coli isolates harboured class 1 and/or class 2 integrons. Class 1/class 2 integrons were detected in 45 E. coli isolates associated with human infections and in 55 E. coli isolates from animal origin. Single class 1 integrons were detected in 78 (34%) E. coli isolates. Combination of class 1 and class 2 integrons were detected in 4 (2%) isolates. Eighteen different genes spread within 11 different gene cassette arrays were observed in class 1 integrons. The most frequent cassette arrangements of class 1 integron were as follows (number of isolates%): dfrA1+aadA1 (30/37%), dfrA17+aadA5 (22/27%) and aadA1 (11/13%). dfrA1+aadA1, dfrA17+aadA5 and dfrA12+orfF+aadA2 gene cassette arrays were found in isolates either of human or animal origin, whereas dfrA5+ereA2, oxa30+aadA1 and dfrA17-cemA gene cassette arrays were found in isolates from human origin only.

Single class 2 integrons were found in 18 (8%) of E. coli isolates and were represented by four different gene cassette arrays. The most frequent cassette arrangement of class 2 integrons was dfrA1+sat1+aadA1 (15/68%) present in human and animal isolates. The plasmid location was confirmed for 8 out of 11 identified class 1 and for 2 out of 4 identified class 2 integrons.

Conclusions: This study is the first report on the prevalence and characteristics of class 1 and class 2 integrons in Lithuania indicating their broad dissemination among human and animal E. coli isolates.

Sulfonamide resistance, caused by sul3 located in unusual integrons, seems to be widespread among bacteria from animals and humans in the community. The aim of this work was to determine the frequency and genetic environment of sul3 gene from clinical Enterobacteriaceae isolates recovered in the last years.

Methods: A collection of 344 Enterobacteriaceae clinical isolates from our institution (1988–2006) which included extended-spectrum (ESBL, n=96)/metallo- (MBL, n=3) β-lactamase producers and non-producers (n=30) was studied. Clonal relatedness was established (PFGE, phylogenetic groups) and antibiotic susceptibility by disk diffusion (CLSI). Genetic platforms were determined based on known sequences by PCR, long-PCR, RFLP and sequencing. Twenty-four sul2-positive strains were selected for conjugation assays. Plasmid analysis included determination of size (S1 nuclease), incompatibility groups, replicases and relaxases (PCR, hybridisation and sequencing) and rep and mob genes of recent published low %CG sul2-plasmids from environmental origin.

Results: sul2 was similarly distributed among E. coli (n=124) phylogroups (A+B1=49%, B2+D=43%) and it was identified in strains with a variety of ESBLs (CTX-M-1, -3, -32, -15, -9, -14, SHV-5, -12, TEM-4, -24, -27, -52) and MBL (VIM-1) and, similarly, in non-producers. sul2 gene was transferable in 19/24 of cases, associated with sulfonamide, streptomycin and/or tetracycline markers. Some (n=12) transconjugants presented more than one plasmid. No apparent association between sul2 gene and specific plasmids was observed. Some strains presented two different sul2-containing plasmids, conjugative (50–380 kb) and non-conjugative (5–12 kb). Most conjugative plasmids belonged to rep type IncB/O, sul2 was found adjacent to ISCR2, entire or truncated (n=48) and to both repC (pRSF1010) and strAB (n=31) genes. Overall, 34% sul2-positive strains presented colicin genes. sul2 was also detected in 5 non-B1 E. coli strains.

Conclusions: Despite scarce use of sulfonamides in humans, the sul2 gene is frequent in clinical isolates. Its presence is related to different plasmids and genetic platforms containing ISCR2 and other resistance genes affecting widely used antimicrobials. Co-selection processes might have fuelled persistence of sul2 gene containing isolates.
were found, three of them identical to those previously described. All of them showed a common region qacH-IS404-sul3. Size of sul3-plasmids varied from 55 to 220 Kb, although those of 100 Kb were the most common and belonged to IncI1 complex (n = 13). Ten strains contained plasmids carrying both sul3 and blaSHV-12. Three of them also produced other ESBLs/MBL (CTX-M-type or VIM-1 together with SHV-12).

**Conclusions:** sul3 was relatively frequent and linked mainly, with human isolates from the community expressing ESBLs and/or MBLs. An association with an epidemic IncI1 plasmid carrying blaSHV-12 is reported.

**P1511 Diversity of Tn402 defective variants associated with class 1 integrons**

A. Navais*, E. Machado, R. Cantón, L. Pérez, F. Baqueri, T.M. Coque (Madrid, ES, Oporto, PT)

**Objective:** Class 1 integrons are Tn402 derivatives frequently associated with mercury transposons. Whole characterisation of class 1 integrons has scarcely been described. A comprehensive analysis of class 1 integrons was performed among representative Enterobacteriaceae isolates recovered in our institution during the last 15 years.

**Methods:** Forty-five Enterobacteriaceae (n = 32 E. coli, n = 8 K. pneumoniae, 3 E. cloacae, 1 S. enterica and 1 K. oxytoca) of clinical (n = 37, 82.2%) and non-clinical (n = 8, 17.5%) origin, producing and non-producing different ESBL (TEM-4, -24, SHV-2, -2a, -5, -12, -13, CTX-M-10, -14) were studied (1988-2003). Analysis of class 1 integrons included characterisation of intI1, 5′CS-3′CS variable region, tniTn402, and screening of sequences orf5, IS1326, IS135 and, IS6100 (PCR simplex and overlapping, sequencing). Association with mercury transposons was searched by screening the presence of merA, tniTn21, tniTn1696 (hybridisation, PCR simplex and overlapping, sequencing).

**Results:** Isolates were classified in Group I (n = 38), showing variable gene cassette content [XVI integron (ln) types were identified]; and Group II (n = 7), positive only for intI1. Four subtypes were established according to the presence of sequences truncating the tniTn402 module: Subtype a) IS1326- (n = 10; In types I, II and VII); subtype b) IS1326 plus IS1353 (n = 3; In types VI and XII); subtype c) IS6100 (n = 14; In types I, III, VI, VIII, IX, XIII-XVI, IntI1+); and subtype d) absence of ISs (n = 11; In types I, II, VII-XI). merA (n = 25/45; 55.6%) was commonly detected among subtypes a, b (mostly associated with Tn21-mer sequences and/or tnpTn21 transposition module), subtype c (linked to tnpTn1696, tnpTn21 or none of them) and less frequently to subtype d (eventually linked to tnpTn1696, tnpTn21). In2 promoter sequence (weak version) was the most frequently identified (72%), although variants In1 (intermediate) and In4 (strong) were also detected. Group I subtypes were mostly identified among ESBL producing isolates but those of group II were absent among ESBL producers.

**Conclusions:** A high diversity of Tn402 variants located in different mercury transposons platforms was associated with identical gene cassette arrays (5CS-3CS). The high variability observed among the genetic platforms suggests recombinitarial events between mobile elements widely disseminated among human Enterobacteriaceae isolates.

**P1512 Antimicrobial resistance of Salmonella strains implicated in 38 community outbreaks in northern Spain**

M. Bances, M.A. González Hevia*, R. Rodicio, C. Mendoza (Oviedo, ES)

**Objective:** To analyze the antimicrobial resistance of Salmonella strains related with community outbreaks recorded in Asturias, Spain, between 2002-2005.

**Methods:** A total of 377 Salmonella isolates related with 38 outbreaks that occurred in institutions (restaurants, hospitals, schools and others) were analysed. Of these isolates, 349 were collected from patients attended in hospitals and recorded in the Public Health Laboratory del Principado de Asturias (PHILPA), the other 28 isolates were collected from food outbreaks associated in the PHILPA. Serotyping and phage typing were determined in the “Centro Nacional de Referencia de Salmonella”, Madrid Susceptibility/resistance was assayed according to CLSI, by the disk diffusion method, using Mueller Hinton agar plates, and disks of ampicillin 10 (Ap), ciprofloxacin 5 (Cip), cloramphenicol 30 (Cm), gentamicin 10 (Gm), kanamycin 30 Km), streptomycin 10 (Sm), nalidixic acid 30 (Nal) tetracycline 30 (Te), sulfadiazine 300 (Su) and trimethoprim-sulfamethoxazole 1.25/23.75 (Sxt). Plasmid analysis was done by the Kado-procedure; presence of integrons of classe 1 by PCR using primers of conserved 3′CS and 5′CS regions; and presence of specific R-genes by PCR using described primers.

**Results:** Twenty nine outbreaks were caused by S. Enteritidis, six by S. Typhimurium and one by S. Infantis, S. Panama and S. Hadar. More than a half of S. Enteritidis were susceptible, whereas near of a quarter part were resistant to ampicillin, encoded by the gene blaTEM-1 (24%), and nalidixic acid (20.7%). Two of the six S. Typhimurium isolates were susceptibles, other two resistant only to tetracycline, and the other two were multiresistant, each with a R-profile: Ap, Te, Su, Cm, S (carrying a class 1 integron, intH) and Te, Su, S, K. Isolates belonging to S. Infantis were susceptible, whereas isolates of S. Panama were nalidixic acid resistant, and isolates of S. Hadar were resistant to Nal, Te, Ap, S. Most of the isolates carried plasmids, some of these corresponding in size to virulence (V) plasmids specific of serotypes Enteritidis (60 Kbp), Typhimurium (90 and 125 kbp, this is a hybrid VR plasmid carrying the integron H).

**Conclusion:** A majority of Salmonella outbreaks were caused by S. Enteritidis, with an increase in the rate of isolates resistant to ampicillin and nalidixic acid, that was also noticed in the last decade. Interestingly one S. Typhimurium strain, causing a nursery outbreak, belonged to an emergent clone carrying a hybrid VR-plasmid.
**P1514** Characterisation of class 1 and class 2 integrons among bacteria isolated from an urban waste water treatment plant in Italy

C. Pellegrini*, G. Celenza, C. Forcella, E. Sacchetti, B. Segatore, P. Bellio, D. Setacci, C. Di Listo, S. Rainaldi, G. Amicosante, M. Perilli (L’Aquila, IT)

**Objectives:** The role of environmental bacteria as a reservoir for antibiotic resistance determinants is still poorly established. The spread of resistance genes is greatly enhanced when they form part of a mobile gene cassette, associated with integrons. The aim of this study was to investigate the presence and distribution of integron-carrying bacteria from a urban waste water treatment plant of L’Aquila city (Italy).

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Integrons (location)</th>
<th>Variable regions</th>
<th>Resistant phenotype</th>
<th>Carbenemases</th>
</tr>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>I(C) 1664</td>
<td>fyr1,aadD</td>
<td>AMP, AMX, AMC, STX</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>I(C) 1664</td>
<td>fyr1, aoa1</td>
<td>AMP, AMX, AMC, STX</td>
<td>-</td>
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<tr>
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<td><em>Acinetobacter baumannii</em></td>
<td>I(C) 1664</td>
<td>fyr1, aoa1</td>
<td>AMP, AMX, PIP, CAZ, STX</td>
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<td>aoa1</td>
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<td><em>Acinetobacter baumannii</em></td>
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<td>-</td>
<td>AMP, AMX, PIP, CTX, CAZ, STX</td>
<td>-</td>
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</table>

Methods: During two years (may 2005-december 2007), 628 Gram-negative bacteria were isolated at different stages of the waste water treatment process, and selected on selective medium supplemented with ceftazidime 6mg/L and imipenem 2mg/L. Resistant bacteria were screened for the presence of integrase genes by colony blot hybridisation. Genotyping of integrase-positive strains was carried out by RAPD analysis. Variable region was investigated by PCR using primers designed to conserved regions of the integron structure, and sequenced. Plasmid profile was performed on selected strains.

**Results:** Overall 40% (251/628) of strains harboured an integron. The predominant organism (37.5%) was represented by *Escherichia coli*. PCR analysis with specific primers for intI1and intI2 genes was performed on 32 isolates that showed different genotype profile. The intI1 gene was detected in 26 out of 32 isolates screened and intI2 gene in 6 out 32 isolates. Sequence analysis of variable regions showed five cassette arrays in class 1 integrons and two arrays in class 2 integrons, encoding for antibiotic resistance determinants, as shown in table 1. Most of the integrons were located in chromosomal DNA, whereas only two integrons were found to be into large plasmids. Several strains contained \( \beta \)-lactamase genes, such as blaTEM-1 gene, blaCTX-M-1-type gene (detected in *Alcaligenes faecalis*), blalMP-22, blalVIM-1 and blalAmpC gene.

**Conclusion:** Our results support the hypothesis that waste water treatment might be an important antibiotic resistance reservoirs and highlight the risk of spreading of harmful gene cassettes through discharges in aquatic ecosystems.

**P1515** Class 1 and 2 integrons determining multidrug resistance to *Acinetobacter spp.* and *Pseudomonas aeruginosa*

E.C. Climaco, D.Y. Takata, M.G. Oliveira, A.L.C. Darini* (Ribeirao Preto, Juiz de Fora, BR)

**Objective:** The main objective of this study was to determine the importance of class 1, 2 and 3 integrons in multidrug resistance phenotypes of *Acinetobacter* spp. and *P. aeruginosa*. In addition, the presence of carbapenemases-encoding genes was investigated.

**Methods:** Firstly, 63 *Acinetobacter* spp. and 149 *P. aeruginosa*, isolated from inpatients of University Hospital of Juiz de Fora, Brazil, from 2000 to 2007, were classified as multidrug resistant (MDR) or non-multidrug resistant (n-MDR). Class 1, 2 and 3 integrons were investigated by specific PCR amplification of intI1, 2 and 3 genes fragments. The prevalence of integrons were compared among MDR and n-MDR groups, and statistical significance was estimated applying Fisher’s exact. Metallo-carbenemase production was also investigated by PCR amplification and sequencing of blalMP, blalVIM and blalSPM genes.

**Results:** 54 (36%) *P. aeruginosa* isolates and 45 (71.4%) *Acinetobacter* spp. were considered MDR isolates. Class 1 integrons were detected in 68 (45.6%) *P. aeruginosa* isolates, 51 of them (75%) were MDR; and in 11 (17.5%) *Acinetobacter* spp. isolates, all of them were MDR. Class 1 integrons association with MDR *P. aeruginosa* and MDR *Acinetobacter* spp. was statistically significant. Class 2 integrons were found in only one MDR *P. aeruginosa* and in 23 (36.5%) *Acinetobacter* spp. isolates. Of them, 20 isolates (87%) were MDR. Class 2 integrons relationship with MDR *Acinetobacter* spp. isolates was statistically significant. Class 3 integrons were detected only in one MDR *P. aeruginosa* isolates. Table 1 shows the association of class 1 and 2 integrons with susceptibility rates to each tested antibiotic. Carbapenemase production was detected in 27 (18.1%) *P. aeruginosa* isolates by amplification and sequencing of blalSPM-1. Of them, 26 isolates were MDR and harboured class 1 integrons.

**Conclusions:** Class 1 integrons were more prevalent than other classes of integron in *P. aeruginosa* isolates, and it shows statistical association with MDR phenotypes, suggesting its importance to confer this resistance profile. SPM-encoding genes were found coexisting with these integrons in *P. aeruginosa*. Class 2 integrons were more prevalent than class 1 ones in *Acinetobacter* spp. isolates, although both classes seem to be determinant to MDR phenotypes in these isolates. Further studies about cassette genes are going to be evaluated to better elucidation about real role of integrons in MDR phenotypes. Supported by FAPESP and CNPq.
Impact of genetic surroundings on the function of integrons

Horizontal gene transfer has produced a large part of the fastly increasing antibiotic resistance among Gram-negative bacteria. Integrons and ISCR elements have contributed to renewal of the mobile assortments of bacteria, suggesting the presence of mechanisms facilitating horizontal gene transfer. The natural transformation might be involved in integron transfer. The aim of this study was to test the hypothesis that natural transformation is one of the mechanisms of integron movement of integrons. Natural transformation is one of the mechanisms that allows bacteria to acquire foreign DNA without plasmid or phage involvement. The aim of this study was to test the hypothesis that natural transformation might be involved in integron transfer. The natural transformation of bacteria has resulted in emergence and dissemination of antibiotic resistance genes and also promoted exchange between clinically important bacteria and microorganisms in the environment. It is increasingly realised that this exchange may be regulated by stress response and that integrons may be organised in larger networks of DNA-pro cessive and istic elements called mobilomes. This study focuses on a few integrons carrying aminoglycoside resistance and antifolate resistance genes and the variable stability of the cassette integrated. The observations support that the level of integrase expression and the genetic surrounding both have strong influence on the operation of the integrons. The aad22 cassette of the small plasmids pMS126 and pMS128 is excised two orders of magnitude more often in the latter than in the former. An assembly line for these elements relate them also to similar elements in IncN-plasmids N3 and pLM020. The formation of a composite element of ES6100 in pMS126 has allowed an integration bypass of a barrier that normally excludes integrons in an IncQ plasmid. We assume that integrons are normally excluded from this highly disseminated class of plasmids.
Skin and soft-tissue infection in patients with solid tumours
Clinical and microbiological analysis of skin and soft tissue infections (SSTI) in patients with solid tumours (ST) and to determine factors leading to treatment failure.

**Objectives:** To describe clinical, microbiological characteristics and outcome of skin and soft tissue infections (SSTI) in patients with solid tumours (ST) and to determine factors leading to treatment failure.

**Methods:** Records of patients with ST and SSTI, cared for at the University Hospital of Heraklion, from 2002 to 2006 were retrospectively studied. Infection's recurrence, need for repeated drainage, and sepsis leading to death were considered as treatment failures.

**Results:** A total of 81 episodes of SSTIs, occurring in 71 patients with ST, had been evaluated. Their median age was 65 years (34–82); 38 (53%) were males. The most common underlying malignancy was breast cancer in 17 patients (24%), followed by colon in 14 (20%), lung in 13 (18%), genital in 10 (14%), head & neck in 5 (7%), and sarcoma in 3 (4%). The remaining 5 (7%) consisted of hepatobiliary, pancreatic and stomach cancer. Most episodes (72; 89%) occurred in non-neutropenics. Cellulitis/erysipelas was the most common clinical presentation (56%; 69%), followed by abscesses (18; 22%), wound infection (5 6%), furunculosis (1 1%) and myositis (1%). Bacterial cultures were possible in 29 (36%) patients and 33 pathogens were isolated. In 5 episodes (17%) more than one pathogens were isolated. Among the microbiologically documented episodes, Gram negative bacteria were isolated in 18 (54.5%), with *E. coli* (7 out of 18; 39%) and *P. aeruginosa* (6; 33%) being the most frequent, followed by *E. cloacae* (2; 11%), *K. pneumoniae* (2; 11%) and *C. freundii* (1; 5.5%). Gram positive organisms were isolated in 14 cases (42%), with *Enterococcus spp* being the most common (6 cases out of 14; 43%), followed by *S. epidermidis* (5; 36%) and *S. aureus* (3; 21%). *B. fragilis* was isolated in 1 episode (3%). Three out of 20 successfully treated patients (15%) and 7 out of 9 who failed (78%) received inappropriate empirical treatment (p = 0.001). Five (7%) patients died due to sepsis. None was neutropenic. Multivariate analysis showed that sepsis on admission (OR 21, 95% CI: 2.97–161.81; p = 0.002) was associated with treatment failure.

**Conclusion:** SSTIs can be life-threatening among patients with ST, even in the absence of neutropenia. Early diagnosis is of utmost importance, since sepsis on admission was proven a significant factor of unfavourable outcome.

**Factors affecting the duration of intravenous antibiotic therapy for cellulitis in an outpatient setting**

**Objectives:** Cellulitis is a common community acquired infection that is increasingly treated with IV antibiotics in an outpatient setting. We analysed patients under the care of our outpatient IV antibiotic service for cellulitis to ascertain factors affecting duration of therapy.

**Methods:** A retrospective review of cases of cellulitis managed by the outpatient IV antibiotic service at our institution between 1st October 2007 and 30th September 2008. Demographic, clinical, laboratory and outcome data were collected. Cases were split into short course therapy (<3 days) or extended course (>7 days). Factors associated with increased duration on univariate analysis were then entered into a multivariate regression analysis. P values <0.05 were considered significant.

**Results:** 98 cases were available for review. 61.2% were male, the mean age was 54.6 years and the mean duration of therapy was 6 days. 28 cases had ≤3 days therapy, 32 had 4–6 days and 38 had >7 days. On univariate analysis (table 1) extended course treatment was associated with higher baseline CRP, ESR, creatinine, longer duration of cellulitis pre outpatient IV antibiotics, lower haemoglobin, increasing age, presence of diabetes and male sex. In the multivariate model, male sex (P < 0.001), increasing age (P = 0.038) haemoglobin (P < 0.001) and CRP (P < 0.001) remained predictive of an extended course.

**Conclusion:** An extended course of IV therapy for cellulitis in an outpatient setting is associated with male sex, increased age, low baseline haemoglobin and high baseline CRP. These factors may be useful to guide frequency of medical review of patients in this setting and may also help with decisions about the nature of intravenous access for antibiotics.
Common pathogens isolated in diabetic foot infection in a rural hospital


Objectives: Foot ulcers are a frequent complication of patients with diabetes mellitus, accounting for up to 20% of diabetes-related hospital admission in previous studies. Secondary infection of these ulcers associated with high morbidity and risk of lower extremity amputation. The aim of the present study was to determine the pathogens isolated from soft-tissue in patients with diabetic foot infections in our hospital.

Methods: 78 patients (hospitalised and outpatients) with diabetic foot infection, 51 (65.4%) male and 27 (34.6%) female with mean age 52±12.7 years, were enrolled in this study. All patients underwent surgical debridement and tissue specimens were obtained by scraping the base of the ulcer with a scalpel or by wound. Bacteriological diagnosis and antibiotic sensitivity profiles were carried out and analyzed using standard procedures. In patients with clinically suspected osteomyelitis, magnetic resonance imaging or bone scintigraphy with white blood cell scanning, were performed.

Results: 102 bacteria were documented microbiologically in this study. The most frequent bacterial isolated were: Staphylococcus aureus in 44 (42.3%) cases, Enterobacteriaceae in 13 (12.5%), coagulase-negative Staphylococcus spp. in 11 (10.6%), Pseudomonas aeruginosa in 11 (10.6%), Streptococcus spp. in 10 (9.6%) and Escherichia Coli in 6 (5.8%) cases. No anaerobes were isolated from the ulcers. Polymicrobial infection was found in 12 (15.5%) cases. 11 (14.1%) patients had combined osteomyelitis and deep soft tissue infection. All Staphylococci were sensitive to vancomycin and teicoplanin, 86.3% to cotrimoxazole and 64.2% to clindamycin. The sensitivity of Gram-negative microorganisms to antibiotics was: 96% to ciprofloxacin, 94% to piperacillin/tazobactam, 84.7% to cotrimoxazole, 69.4% to amoxicillin/clavulanic. MRSA was isolated in 4 samples. Bacteria were obtained from patients with both osteomyelitis and deep soft tissue infection had statistically high rate resistance to antibiotics than bacteria were isolated from patients with soft tissue infection only, p < 0.001.

Conclusions: Gram-positive cocci were the most common causes of diabetic foot infection in our area (up to 50%). Knowledge of microorganism panel in patients with diabetic foot infection in our hospital may help us for effective empirically treatment until culture results and susceptibility data of ulcer lesions be known.

Diabetic foot ulcers: a bacteriologic study of 193 patients

S. Bakas*, E. Panagiotopoulos, A. Spathi, A. Lykas, F. Petropoulou, E. Logothetis, K. Gerolymatou, E. Kouskouni (Athens, GR)

Objectives: Diabetic foot ulceration and infection represent an important cause for hospitalisation, enhancing the risk for subsequent amputation. Usually these infections are polymicrobial in nature so correct and early isolation and identification, as well as prompt initiation of appropriate antibiotic therapy are important steps toward a successful outcome. This study was undertaken to identify the pathogens associated with diabetic foot infections in our hospital.

Methods: A total of 193 consecutive patients were included in the study during the period November 2006 to November 2008. Only diabetic patients presenting with foot infection and who did not receive antibiotics for the past 30 days were included in the study. Clinical specimens collected from patients were inoculated onto appropriate plates for standard aerobic and anaerobic cultures and incubated at 37°C for 24 h and 48 h, respectively. A Gram-stained smear from the specimen was examined under microscope to obtain valuable information about the types of microorganisms present. The isolated pathogens were identified using the automated system Vitek 2 (BioMerieux, France).

Results: The mean age of the patients was 61.4 years (range 24–78) with 109 (56.5%) of them being males and 84 (43.5%) females. A total of 368 pathogens were isolated, resulting in an average of about 1.9 microorganisms per sample. We isolated 127 aerobic Gram-positive cocci representing 34.5% of all pathogens, 179 (48.7%) aerobic Gram-negative rods, 61 (16.7%) anaerobic bacteria and only 1 Candida sake (0.01%) from our samples. Staphylococcus aureus was more frequently isolated among the Gram-positive cocci (46.1%), Proteus mirabilis was more frequently isolated among the Gram-negative rods (31.4%) and Bacteroides species represented the 91.9% of all anaerobic bacteria isolated. One hundred and four (53.9%) patients had one microorganism, 38 (19.7%) had 2 pathogens, 26 (13.5%) had 3, 20 (10.4%) patients had 4 pathogens and 5 (2.5%) patients had 5 pathogens isolated from their foot ulcers.

Conclusion: In our study group diabetic foot infections were mostly monomicrobial. The most frequently isolated microorganisms from the ulcers were S. aureus, P. mirabilis and Bacteroides species. Constant awareness of isolated pathogens in these infections is essential for the optimal management and a successful outcome of diabetic foot ulcers.
of spinal pain unrelieved by rest or fever and spinal pain on physical examination, together with one or more imaging techniques showing data compatible with VO following the criteria of Dagirmanjian et al. All patients received specific antibiotic treatment for two months, being intravenous for at least four weeks. Patients with large soft tissue masses, cord compression, highly destructive lesions or those who suffered a therapeutic failure, were also treated surgically.

**Results:** Of 267 patients with PVO, 51 (19.1%) had GNBVO and of them 12 (23.5%) were PAVO. Of the patients with PAVO, 7 (58.3%) were male and 5 (41.7%) female (mean age, 59.1 ± 13.6 years). Five patients (41.7%) had previous spinal surgery, 4 (33.3%) had skin or soft tissue infection and 3 (25%) were diabetic. The vertebral level involved was lumbar in 7 cases (58.3%) and thoracic in 5 (41.7%). The median time to diagnosis was 12 days (range; 4–120 days). Five patients (41.7%) had neurological deficits. Paravertebral or epidural masses were detected in 6 cases (50%). Five patients (50%) required surgery. One case was lost in follow-up. Of the 11 remaining cases, all cured, although two (18.1%) had severe functional sequels.

Previous spinal surgery and skin or soft tissue infection were significantly more frequent in PAVO, whereas urinary or gastrointestinal infection was more frequent in OBGNVO. The mean hospital stay was significantly higher in PAVO group, 72.7 ± 33.19 days versus 42.6 ± 25.2 days respectively. We did not find other clinical, biological or radiological differences between both groups.

**Conclusions:** P. aeruginosa is a frequent cause of GNBVO. In patients with VO, previous spinal surgery and skin or soft tissue infection should make considering P. aeruginosa as a possible aetiologic agent. With an appropriate treatment, the prognosis of PVO does not differ of others VO caused by GNB.

**Background:** Chronic osteomyelitis (CO) is a treatment challenge for ID specialists as the optimal regimen and treatment duration are still not fully established.

**Methods:** We retrospectively analyzed 144 CO cases, documented upon clinical, radiological, histology and available microbiological data. Cases were retrieved from a data-base registry of all patients with bone and joint infections followed in our Department during the last decade. Only pathogens isolated either from intra-operative or bone aspiration samples were included. CO cases treated either empirically (ET) or upon microbiological documentation (MT) were comparatively assessed. Duration of treatment was individualised. Predictors of treatment failure were analyzed. Treatment failure was the absence of clinical, radiological and microbiological resolution of the infection.

**Results:** From 144 CO eligible cases, 105 (73%) were male. Median (IQR) age was 48 (31–63) years, and median (IQR) duration of treatment was 6 (4–10) months. Surgical debridment was performed in 90/144 cases (65.3%). Distribution of aetiology in the MT group (117/144, 81.2%) was as follows: MRSA (21.3%), MSSA (22.2%), CoNS (12.8%), P. aeruginosa (11.1%), other Gram negative (12.8%), Gram positive (6%) and polymicrobial infection (9%). In the ET group ciprofloxacin – along with either rifampicin or clindamycin – was administered in 17/27 cases (63%), while glycopeptides only in 3/27 ones (11.1%). Patients in the ET group were older (>60 years, p = 0.05), with more comorbidities (p = 0.007) less often sinus tract (p = 0.003), more clindamycin use (p = 0.01) and less surgical debridment (p = 0.01) compared to patients in the MT group. No difference in treatment duration (p = 0.9), side effects (p = 1.0) and rates of treatment failure (37/117; 31.6%, vs 6/22, 22.2%, p = 0.5) was assessed between MT and ET group. Overall, side effects (n = 38/144, 26.3%) were mostly related to SMX-TMP administration (n = 16). Regarding predictors of outcome, only fever (OR 2.8, 95% CI 0.9–8.3, p = 0.03) and the relatively short (<3 months) duration of treatment (OR 1.8, 95% CI 1.0–3.0, p = 0.05) were found to predict treatment failure.

**Conclusions:** Treatment duration more than 3 months appears to be the most important factor related to the remission of the infection regardless of ET or MT antimicrobial treatment of CO. Cautious choice of an empirical combined antimicrobial treatment in a selected population with CO may lead to a favourable clinical outcome of the infection.
Clinical presentation and outcome of septic arthritis


1. Cooper C, Cawley M. Bacterial arthritis in an English health district: elevated mobility, specially in febrile patients. There were not changes on bacterial aetiology according with the inflammatory symptom were more prevalent than infectious manifestations. 3.

Conclusions: Other predictors of morbidity and mortality.

Other predictors of morbidity and mortality.

and/or positive blood sample cultures. Osteoarthicular involvement was defined by inflammatory signs in peripheral joints with radiologic alterations and/or lumbar and sacral vertebrate magnetic resonance image in for patients who had lumbaro of brucellosis.

Results: Sixty-five patients (42%) had osteoarthicular involvement (55.4% male, 44.6% female). The mean age was 45.8 ± 18 years. Sacroiliitis was the most common involvement (n: 39, 60%), followed by spondylodiskitis (n: 27, 41.5%), peripheral arthritis (n: 10, 15.4%), bursitis (n: 1, 1.5%). In addition, there were 11 (17.5%) patients with more than one site of involvement: nine had concomitant sacroiliits and spondylodiskitis, one had sacroiliits and peripheral arthritis, and the remainder sacroiliits and bursitis. Spinal epidural abscess was found seven cases. Sacroiliits has been determined symmetrical 82% rate. Lumbar spondylodiskitis was the most common involvement among the spondylodiskitis (n:25, 92.5%). All of the cases were treated doxycycline and rifampicin or streptomycin, rifampicin and doxycycline according to their clinical progress at least 3 months.

Conclusion: Brucellosis should be included in the differential diagnosis of any patient with arthralgia or symptoms of sacroiliits and spondylodiskitis especially where this infection is endemic countries such as Turkey.

P1520 Clinical presentation and outcome of septic arthritis diagnosed in a community hospital

A. Leach, C. Amador*, J. Rosas, M. Lopez, C. Martinez, M. Gil, F. Pasquau (Villajoyosa. Alicante, ES)

Objective: To describe the aetiology, clinical presentation, treatment and prognostic factors of septic arthritis (SA) in adult patients diagnosed in our hospital.

Methods: Retrospective analysis of the records of patients diagnosed of SA according the database of Admission Service and review of the cultures of synovial fluid in the Microbiology department from January 2002 to November 2008.

Results: 53 episodes of SA were included on the period of study. According to the modified criteria of Newman and Cooper Cawley (1), 68% of these patients were male, the mean age was 53 years (16–91 years) and 50% had underlying diseases, 20% joint disease. Clinical manifestations were pain (95%), joint swelling (90%) and fever (45%). Only 6.7% had general malaise. The knee was the most frequent joint affected (58%) followed by the hip (10%). Only one patient had polyarticular involvement. The most common causative organisms were S. aureus (37%) followed by Streptococcous spp. (8%) and Gram-negative bacilli (7%). 8.3% of the cultures of synovial fluid were Polimicrobial. Bacteremia was documented in 10% of patients but blood cultures were drawn just in one third of cases. Empiric antibiotic therapy was considered correct in 46% of the cases. 39% of patients received NSAIDs and 6.8% were treated with steroids. Surgical drainage and continuous lavage were done in 33 (55%) patients and 17% had repeated punctures joints. 38% of patients had functional sequelae and 4 patients died during admission, two of them was related to the infection. Poor outcome was significative related with febrile presentation (p < 0.04). There were no other predictors variables of morbs-mortality.

Conclusions: 1. Half of our patients had underlying diseases. 2 Inflammatory symptoms were more prevalent that infectious manifestations. 3. There were not changes on bacterial aetiology according with the literature 4. An optimal combined treatment is desirable to improve the elevated mobility, specially in febrile patients.


P1530 Septic arthritis caused by anaerobic bacteria

M.D. Rojo-Martin*, C. Miranda, E. Torres, T. Sabalete, P. Muzzelas (Granada, ES)

Objectives: Anaerobic bacteria are uncommon pathogens in septic arthritis. The objective of this study was to review acute arthritis due to anaerobic bacteria in patients without prosthetic joint implants diagnosed in the University Hospital Virgen de las Nieves (Granada, Spain) in the last ten years.

Methods: From January 1999 to December 2008 we studied in our service 1735 synovial fluid samples, analyzed by Gram stain, aerobic and anaerobic culture, and in addition, enrichment in broth culture. Cases included were: patients without prosthetic joint implants from whom anaerobic bacteria were detected in the synovial fluid and whose clinical data were compatible with septic arthritis.

Results: During the study period, we detected two cases of septic arthritis by anaerobes. The first case took place in December 2002 in a 23-years-old woman, with a previous diagnosis of pharyngitis. The affected joint was the left ankle, F. necrophorum was isolated in blood and synovial fluid cultures. The outcome was favourable after metronidazol therapy. The second case took place in October 2008 in a 3-years-old boy, with previous traumaism in the left knee. He had no wound nor haematoma in the traumatism area. F. nucleatum was isolated from the first synovial fluid sample, and in another one, it was detected only by universal PCR and subsequent sequencing and phylogenetic analysis of the PCR products. The patient needed arthrothomy and parenteral therapy with imipenem and metronidazol; the patient was discharged with oral clindamycin and the outcome was satisfactory.

Conclusions:

- The incidence of septic arthritis due to anaerobic bacteria was very low, in our series, it was caused by Fusobacterium spp.
- Arthritis caused by anaerobic bacteria is very rare in childhood, however, one of the two cases of arthritis by anaerobes in our series was due to F. nucleatum, which took place in a boy.
- Some anaerobic bacteria are difficult to recover by culture. In cases with suspicion of septic arthritis and negative cultures, PCR could be an important tool to reach a diagnosis.

P1531 Risk factors for lower extremity amputation among diabetic patients hospitalised for a foot infection


Objectives: Lower extremity amputation (LEA) is one of the most feared complications of diabetes, and infection is the chief precipitating cause. We investigated the demographic, clinical, and microbiological characteristics of diabetic patients hospitalised for a foot infection, and attempted to identify, and develop a model for, risk factors for LEA.

Methods: Using the Cardinal Research Database we identified diabetic patients admitted to any of 97 hospitals from 2003 to 2007 for a culture-proven lower extremity skin or soft tissue infection (SSTI). We compared various characteristics of patients who underwent a LEA with patients who did not.

Results: We identified 3018 eligible patients, 647 (21.4%) of whom underwent LEA. The site of amputation was toe in 43.6%, foot in 20.9% and leg in 35.5%. Only 40 patients died (1.3%), 37.5% of whom underwent LEA. Mortality was significantly higher for patients with a leg (major) amputation compared to a toe/foot (minor) amputation (3.9% vs 1.4%, p < 0.05). The illness severity was in the top 2 quartiles for 80.2% of patients with LEA vs 41.6% for patients without LEA, and 91.3% for patients with major vs 74.1% for patients with minor amputations. The most frequent single pathogen was S. aureus, comprising 22.2% of all cultures; 36.3% of these isolates were methicillin-resistant (MRSA). Overall, 57.2% of infections were polymicrobial. LEA patients were more likely to have polymicrobial infections than patients without LEA (64.1% vs 55.3%, p < 0.0001). Patients with major amputations were more likely to have polymicrobial infections that included MRSA (12.6% vs 7.4%, p < 0.05) or Pseudomonas (14.8% vs 8.2%, p < 0.01) than patients with a minor LEA. Significant independent risk factors for LEA are shown in the table. The resultant model showed very good discrimination and fit (c-statistic 0.76, Hosmer-Lemeshow p > 0.18).

Conclusions: Among diabetic patients hospitalised for a LE SSTI over 20% underwent LEA and over a third were major. LEA was associated with increased illness severity and mortality. S. aureus was the most
frequent pathogen, but most infections were polymicrobial. Significant (p < 0.0001) covariates associated with LEA included surgical site infection, vasculopathy, hypoalbuminaemia, leukocytosis, coagulopathy, and polymicrobial infection. Using this large database we developed a model that appears to accurately estimate LEA likelihood based on easily available demographic, clinical and laboratory data.

Epidemiology and clinical aspects of infections

**Actinobaculum spp.: clinical observation of 19 cases**
C. Béguelin*, L. Rotto, M.I. Tritten, H. Siegrist, D. Genné (La Chaux-de-Fonds, CH)

**Objectives:** Actinobaculum spp. are new species that have so far been isolated from human blood, urine and pus. Their importance has probably been underestimated and the different species underdiagnosed until today, as the laboratory needs to search them actively. The aim of this study is to examine their clinical relevance.

**Methods:** This retrospective study takes into consideration all known cases of Actinobaculum spp. infections identified since 2004 in the canton of Neuchâtel (169 000 inhabitants), Switzerland. Strains were cultivated and isolated in the bacteriology laboratory in its routine procedure. Identification usually included a API 32 A gallery (bioMérieux) and 16S RNA gene sequencing.

**Results:** Twenty positive samples could be found in 19 patients: (11M/8F) of all ages (16–91), 10 urine (50%), 6 blood (30%), 1 blood and urine (5%), and 3 pus (15%). 12/13 (92%) cases of urinary tract infection (UTI) had an underlying pathology of the genitourinary tract. When urine cultures were positive for Actinobaculum spp., leucocytes were found in all samples but nitrite tests were mostly negative [6/7 (86%)]. All samples showed Gram positive rods. Onset of concordant treatments were delayed by sensitivity of the commonly used antibiotics by 1–2 weeks (weeks 9–11). Fifty percent of the cases were treated as outpatients and 18/19 (95%) had a favourable outcome.

**Conclusion:** To our knowledge, this is the largest series published to date.

In case of leukocyturia with a negative nitrite test but presence of Gram positive rods, in patients with an underlying genitourinary tract pathology, Actinobaculum spp. should specifically be searched instead of considering clinically irrelevant colonisation by Corynebacteria. This infection is probably much more common than previously thought.

**Community-acquired Stenotrophomonas maltophilia infections: a systematic review**
M. Falagas, A. Kastoria*, E. Vouloumanou, G. Dimopoulou (Athens, GR)

**Objective:** Stenotrophomonas maltophilia is a pathogen that causes infections mainly in immunocompromised patients. However, community-acquired S. maltophilia infections have been occasionally reported. The aim of this study is to collect and evaluate the available published data referring to community-acquired S. maltophilia infections.

**Methods:** We searched PubMed, Cochrane Library, and Scopus for articles providing data for patients with community-acquired S. maltophilia infections.

**Results:** Eight case series and 23 case reports (involving 77 and 26 patients with community-acquired S. maltophilia infections, respectively) were regarded as eligible for inclusion in our review. Regarding the 7 case series and 23 case reports, 14 patients with community-acquired S. maltophilia infections were included in the identified case series, 45 had bacteraemia, 6 ocular infections, 5 respiratory tract infections, 4 wound/soft tissue infections, 2 urinary tract infections, 1 conjunctivitis, 1 otitis, and 1 cellulitis; data were not reported for the remaining 12 patients. Comorbidity (such as malignancy, HIV infection, prior hospitalisation) was common. Data included in the 8 case series regarding the outcome of infection were limited. From the 26 patients with community-acquired S. maltophilia infections reported in the case reports, 22 were cured from the infection, whereas 4 of 26 patients died; 1 death was attributed to septic shock due to S. maltophilia.

**Conclusion:** Several publications report patients with community-acquired S. maltophilia infections; the majority of them refers to patients with some kind of comorbidity. Physicians should be aware that S. maltophilia infections are not restricted to hospitalised patients.

**Seasonal patterns of invasive Streptococcus pyogenes disease in the Northern hemisphere**
T. Lamagni*, G. Tyrrell, M. Loughran, T. Siljander, O. Lytikainen, J. Vuopio-Varkila, C. Van Beneden, D. Martin, A. Efstratiou (London, UK; Edmonton, CA; Helsinki, FI; Atlanta, US; Porirua, NZ)

**Objectives:** To compare the seasonal patterns of invasive Streptococcus pyogenes disease among four countries in two northern hemisphere continents on order to evaluate the degree of congruence in timing and magnitude of peaks and troughs in incidence.

**Methods:** National S. pyogenes surveillance data were extracted for Canada, Finland, UK (England, Wales, Northern Ireland) and from ten USA sites for the period 2004–07. For Canada, the UK and USA isolates from all sterile sites were included whilst for Finland just blood and CSF isolates were available. Weekly counts were analysed for each country, with sub-analyses for Western and Eastern Canada. Data were smoothed using six-week moving averages. Seasonal patterns were compared both between countries and to published influenza surveillance data.

**Results:** Comparison of invasive S. pyogenes data between three of the countries identified broadly similar patterns in Canada, the UK and USA of winter/spring peaks and autumn troughs, although seasonal increases started on average 8–10 weeks earlier in S. mutans (weeks 32–43) and the USA (weeks 34–43) than in the UK (weeks 45–51). Seasonal patterns in western Canada were typically several weeks earlier than in Eastern Canada. Winter peaks in Finland were much less pronounced than those in Canada, UK or USA and tended to start earlier. Unlike the other countries, a distinct midsummer influx could be identified in Finland; 21% of all cases in Finland occurred during June and July, compared to <16% for the others (Chi sq. (1 df)=19.71; p < 0.001). Based on the analysis of six-week moving averages, the magnitude of the seasonal peaks were broadly similar in Canada, UK and USA, with the incidence increasing by a magnitude of 2 to 3 on average compared to the annual nadir. In all four countries, the start of the winter influx in S. pyogenes disease preceded the start of the influenza season in most years, although the peak season for both infections overlapped.

**Conclusion:** Seasonal patterns of invasive S. pyogenes infection showed a high degree of congruence between Canada, the UK and USA.
District midsummer peaks were evident in Finland, possibly related to the widespread custom of retreating to summer cottages and the accompanying outdoor activities which could increase skin trauma and exposure to biting insects. Influenza appears to have a limited influence on the initiation of winter increases in S. pyogenes disease but may contribute to later winter peaks.

**[P1535]** The epidemiology of botulism in Romania

D. Dobre, R. Moroti*, A. Hristea, V. Arama, D. Lemeni, M. Sisiroi, A. Luca (Bucharest, RO)

**Objectives**: The aim of this study is to establish the incidence of botulism and the dominant type of the botulinic toxin in Romania.

**Methods**: This is a retrospective analytical study based on information provided by the Cantacuzino National Microbiology Institute (INCDECMIC), The National Centre of Informatics of the Health Department (CNOASIIDSB) and by the Bals National Infectious Diseases Institute (INBI) – Bucharest.

**Results**: In a 7 years period (2000–2007) the total number of cases according to the CNOASIIDSB was 181. INCDMIC confirmed the presence of the botulinic toxin in 92 biological samples received in the same period. Of these 92, 55 tested positive for type B toxin, in 36 cases the type of the toxin couldn’t be identified because of insufficient amount of serum for serotyping, and one sample was positive for type E toxin (in 2007, related to fish products). The disease had a sporadic evolution, most frequently appearing in familial outbreaks, usually associated with the consumption of home prepared meat products. Most cases were reported from the western part of the country (116) and the fewest cases were in southern Romania (26 cases). The average number of cases was about 22/year. The highest incidence was reported in 2007 (0.18/100000), with 38 cases, the most affected being the western areas, almost half of them related to industrially canned Romanian meat products (liver paste and canned fish). The lowest incidence was recorded in 2002 and 2006 (0.06/100000).

**INBI** reported 10 cases in 2007. All patients presented diplopia, palpebral ptosis, midriasis, xerosis and constipation. Nine patients presented urinary retention (severe in 2 cases) and 5 out of 10 developed respiratory failure, one patient requiring tracheostomy.

**Conclusions**: In Romania, type B botulinal infection was widely spread between 2000–2007, the ratio being 55:1 – B:E which explains the less severe forms of the disease due to the predominantly autonomic nervous system involvement in type B botulism. From an average of 20 cases per year during 2000–2006, the incidence increased rapidly (2 fold in 2007). The incidence is high in the western and central regions, sparing the southern area. Most cases occur from small familial outbreaks due to home prepared meat products, except in 2007, when industrially canned products were involved.

**[P1536]** Acute diffuse bacterial external otitis in primary care

St. Fokas*, F. Arapis, Sp. Fokas, D. Rebelou, M. Dionysopoulou (Sparta, GR)

**Objectives**: Acute diffuse bacterial external otitis (AEO) is an infection of the skin of the cartilaginous portion of the ear canal and is commonly seen in all age groups in the primary care setting. We aimed to isolate, characterise and obtain susceptibility profiles of bacteria and fungi from the external auditory canals in adults patients with AEO and to define correlation (if any) of pathogens with Senturia classification.

**Methods**: Specimens were collected from the external canals of 93 patients who visited for the first time the outpatient otolaryngologist department of our hospital with symptoms of AEO, during a 3 years period (2005–2007). AEO was clinically diagnosed and the Senturia classification was done by the otolaryngologist. Patients in the Senturia class IIa and higher were eligible for the study. Conventional methodology for culture and identification to the species level was used and susceptibility tests were performed by Vitek 2 system. Statistical analysis was done by using Fisher's exact test.

**Results**: A total of 85 (91%, 85/93) specimens were found positive for pathogens. Single organism growth was occurred in 67 (79%) samples and two organisms were isolated from 18 (21%) samples. The most common isolates were P. aeruginosa (43%), S. aureus (17%), Candida albicans (15%), Proteus spp. (7%), Streptococcus pyogenes (6%), Haemophilus spp. (4%), Klebsiella oxytoca (3%), Vibrio alginolyticus (2%), Streplococci Group C and G (2%) and E. coli (1%). A total of 3 (17.5%, 3/17) MRSA strains were recovered and P. aeruginosa ciprofloxacin and gentamycin resistance rates were 9% and 13% respectively. No cases of malignant (necrotising) external otitis were diagnosed. No statistical association between specific pathogen and Senturia class was found.

**Conclusions**: P. aeruginosa was the most frequent bacterial species isolated and did not present association with Candida spp. Polymicrobial nature of AEO was established in one-fifth of the patients and Candida spp. was found more common than the other pathogens in these cases. Clinical signs and symptoms as they were classified using Senturia class were not associated with any specific pathogen. Although the susceptibility tests are not used for topical use of antibiotics our data suggests that local antibiotic resistance patterns should be considered since MRSA strains and ciprofloxacin-resistant P. aeruginosa were isolated.

**[P1537]** Epidemiology and drug resistance pattern of cholera outbreak in summer 2008 in Iran

M. Rahbar*, M. Zahraee, A. Omidkarnia, M.T. Afshani, M. Glami, H. Gholami, P. Islami, M. Mohammad Zadeh (Tehran, IR)

**Objective**: Cholera is an endemic disease in Iran. The aim of this study was to determine epidemiology and antimicrobial susceptibility patterns of Vibrio cholerae O1 biotype EL Tor in summer of 2008 in Iran

**Methods**: Stool samples were collected from patients suspected to have cholera admitted to hospitals and clinics. Specimens examined by conventional bacteriological methods. All isolates were sent to cholera reference laboratory for confirmation, stereotyping and susceptibility testing. Antimicrobial susceptibility testing was performed by disk diffusion methods as recommended by Clinical laboratory standard Institute (CLSI). Demographic data collected from questioner forms. The antimicrobial drugs tested included Ampicillin (AM), Co-trimoxazole (SXT), Ciprofloxacin (CI), Tetracycline (TC), Erythromycin (EM), Cholaramphenicol (C), cefoxime (CXM) Furazolidone (F). Nalidixic acid (NA). The E-test MIC method used for detection of minimal inhibitory concentration (MIC) for erythromycin.

**Results**: In total 220 patients diagnosed clinically and laboratory confirmation to have cholera Of 220 cases 199 serotypes were inaba and susceptibility tests were performed by Vitek 2 system. Statistical analysis was done by using Fisher's exact test. No patients were male and 71 (32%) were female. 129 (59%) of patients were male and 71 (32%) were female. 129 (59%) of patients had Iranian nationality, 79 (36%) were from Afghanistan and 12 (5%) from Pakistan. All isolates were resistant to co-trimoxazole, nalidixic acid, furazolidone, and intermediate to chlamphencicol. All isolates were susceptible to tetracycline, ciprofloxacin, and erythromycin. MIC ranged 1–1.5 μg/ml for erythromycin. The antimicrobial results and pulse field gel electrophoresis (PFGE) showed that all isolates had the same epidemiology and susceptibility patterns.

**Conclusion**: Our study reveals that in recent outbreak caused by V. cholerae EL Tor serotype Inaba were the predominant serotype. All isolates were resistant to co-trimoxazole, nalidixic acid and furazolidon.
**P1538** Peritonitis in continuous ambulatory peritoneal dialysis: five-year experience in our hospital


**Objectives:** Peritonitis is the most important and life-threatening complication of continuous ambulatory peritoneal dialysis (CAPD). The aim of this study was to evaluate epidemiological, clinical and laboratory findings, microbiological aspects, treatment approach and outcome of patients with CAPD-related peritonitis.

**Methods:** A total of 89 patients, 51 (57.3%) female and 38 (42.7%) male with mean age of 59±16 years, with CAPD-related peritonitis were enrolled in this retrospective study. Demographical data, clinical, laboratory and physical examination findings of patients were registered. Peritonitis were diagnosed by standard clinical and laboratory criteria (abdominal pain, cloudy dialysate fluid, white blood cell count >100/ml of effluent). Gram stain and cultures were obtained from each patient. Identification and susceptibility testing were performed by Vitek 2 automated system (bioMerieux, France).

**Results:** 104 episodes of peritonitis were detected in a five-year period. 12 (13.8%) patients had more than one episode of peritonitis. The most frequent clinical signs and symptoms were: cloudy dialysate fluid, rebound tenderness, fever, nausea/vomiting in 87 (83.6%) cases. Periperal leukocytosis, elevated C-reactive protein and erythrocyte sedimentation rate were detected in 60.6%, 82.7% and 87.5% cases, respectively. The WBC count of peritoneal effluent ranging from 187 to 6950/ml. Gram stain was positive in 28 (26.9%) effluent. The mortality rate was 2.2%. Identities were performed with the monospecific A and M antisera. Antimicrobial susceptibility will be helpful for earlier and targeted treatment. The mortality rate was 2.2%.

**Conclusions:** CAPD-related peritonitis is still remaining a serious complication of CAPD and coagulase-negative staphylococci are the leading cause of peritonitis. Identities are performed with the monospecific A and M antisera. Antimicrobial susceptibility will be helpful for earlier and targeted treatment.

**P1540** Are measles still a problem? Istanbul, Turkey data

G. Sengoz*, T. Colakoglu, T. Ersoy, N. Kazgunkaya, E. Celikkok, M. Bakar (Istanbul, TR)

Measles is an infectious disease documented since the early years of 20th century in Turkey. Measles was included to be reported diseases list in 1930 and nine months old infants were begun to be vaccinated since 1970 as National Vaccination Programme requested. As a conclusion of this strategy, an obvious decline of the disease incidence was observed in last 20 years. Since 2006, measles vaccine has being applied as MMR to 1 and 7 years old children.

Comparison of annual measles incidences between 1951–2005 Health Ministry of Turkey started a “Measles Elimination Programme” in 2004, by this programme Ministry envisioned to eliminate measles by 2010 year and with this goal began to use probable and confirmed case definitions.

As an important branch of this programme Istanbul Health Directorate provided a 96% vaccination rate in “School Vaccination Days” in the meantime 1,485,125 primary school children vaccinated in 2003 in Istanbul metropolis which has 13 millions of population and 210,000 infants under 1 year. In 2005 “Measles Vaccination Days”, 1,338,823 children in 0–6 age group and 6–14 age group not attending to school have been vaccinated and 94% vaccination rate was reached. Besides every year intense effort is spent to get vaccination rates 95% and over. By carrying out measles vaccination campaign 18,219,757 children have been vaccinated and 94.6% immunisation rate was reached.

After the last case peak of 3,250 patients in 2001, measles elimination programme rapidly concluded and since June 2006 only one measles case was reported in Istanbul. The case reported in October 2008 is a 27 years old woman who traveled to Thailand 3 weeks before and accepted as a foreign source case.

Measles elimination in Istanbul can be declared as already provided as a consequence of determined and effective realisation of measles vaccination campaign which was planned to get by 2010 at first.

**P1539** Epidemiological changes in the human brucellosis

E. Vamvakas, P. Sidra, A. Kafiantzi, A. Iftantidou, A. Kansouzidou* (Thessaloniki, GR)

A significant increase of brucellosis cases in animals and humans has been observed in Greece over the last years. The purpose of this report is the epidemiological study and the evaluation of the identification and typing of Brucella strains isolated from blood cultures of patients admitted to the Infectious Diseases Hospital during the years 2003–2008. The BACTEC 9050 system and BACTEC PLUS-A Aerobic/F bottles were used for the isolation of Brucella strains from blood cultures. The identification and typing of the strains were based on conventional biochemical characteristics, lysis by the Tb phage and agglutination reactions with the monospecific A and M antisa. Antimicrobial susceptibility testing was performed by disk diffusion method and all strains were found susceptible to all antibiotics. The appearance of positive results in blood cultures with the BACTEC system ranged from 2.5 to 5 days. It was found that all Brucella strains were B. melitensis (they were not lysed by the Tb phage) and out of them, 16 strains were identified as B. melitensis biotype 1 (8.5%), 41 strains as B. melitensis biotype 2 (21.7%) and 132 strains as B. melitensis biotype 3 (69.8%). A correlation of these strains to the Brucella strains isolated from humans in previous years (1970–2008) was attempted. The B. melitensis biotype 2 caused illness in the humans almost exclusively, but from 1996 a remarkable increase of the biotype 3 was recorded. From 2001, the proportion of isolation of B. melitensis biotype 3 strains was increased considerably, but a simultaneous increase of biotype 1 and elimination of the biotype 2 was observed. The study of the strains that were isolated at time period 2003–2008 revealed further increase of the biotype 3 as opposed to the previous years. Results of this study showed that B. melitensis continues to be the major agent of human brucellosis in Northern Greece, with the biotype 3 as the most frequent. These findings signal epidemiologic changes in the appearance of illness.

**P1541** Epidemiological and clinical features regarding *Rickettsia conorii* infection in Romania

A. Hristea*, R. Serban, M. Racu, D. Badescu, M. Apostol, A. Streinu Cervel, A. Rafila, V. Aruma, R. Morotti (Bucharest, RO)

**Objectives:** presenting the epidemiological and clinical aspects of *Rickettsia conorii* infection in Romania between 2000 and 2008.

**Methods:** Epidemiological descriptive study of Mediterranean spotted fever (MSF) cases reported in Romania during 2000–2008 presenting the clinical features of cases admitted to INBI “Prof Dr Matei Balș”. The
inclusion criteria were the presence of at least three of the following: fever, maculopapular rash, eschar, history of tick bite/contact plus positive serology (indirect immunofluorescence reaction). The sources of the data are the centralised reports from the National Center for Surveillance and Control of Communicable Diseases Bucharest, case enquiries and charts of the patients admitted between 2000 and 2008.

Results: The incidence for the whole country varied from 0.4/100,000 (2006) to 2/100,000 (2001). All the cases reported during 2000–2008 were from the southern part of Romania. The counties with the highest incidence were Constanța (44.2/100,000 in 2001), Tulcea (39/100,000 in 2002), and Bucharest (3.15/100,000 in 2002). The patients were mainly from urban areas (85.5%) the infection being related to recreational activities. Most patients (83%) acquired the infection between June and September. We have also collected clinical data from 270 patients admitted to the National Institute of Infectious Diseases "Matei Bals" of which 56.7% were women and 43.3% men, with a mean age of 47 years. The painless tick bite was often-unnoticed (46.6% of patients). The clinical features consisted of: fever 96.6%, rash 97.4%, eschar 46.8%, myalgia 42.6%, arthralgia 15.9%, headache 39.3%, neurologic manifestations 2.9%. Laboratory tests revealed: ALT >N in 103/244 (42.2%), of which >2N in 29/244 (11.9%); WBC >10,000/mm3 68/269 (25.3%), PLT <150,000/mm3 116/269 (43.2%), fibrinogen >400/mg/dl in 140/194 (72.2%). There were two fatal cases during this period but none among the patients admitted to our institute.

Conclusion: The sporadic nature of cases demonstrates the endemcity of the disease in Romania. Present ecological and climatic changes lead to the widening of previous endemic areas, thus contributing to an increasing number of infections especially the subclinical ones. The geographic distribution of MSF widened to areas that are not on the shores of the Black Sea. MSF should be considered in the differential diagnosis of any traveler returning with fever, history of tick bite, rash, and/or eschar (tache noire) from endemic areas.
Performance of the Gen-Probe APTIMA COMBO 2® assay on the Panther analyzer

C. Clark*, M. Vi, J. Pham, C. Nguyen, T. Trsic, L. Villegas, A. Worlock (San Diego, US)

Objectives: The objective was to evaluate the analytical performance of the Panther; a fully automated molecular diagnostic analyzer under development at Gen-Probe and compare it to the TIGRIS® DTS® Automated Analyzer.

Methods: A six-member panel was made by spiking rRNA from Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) into Specimen Transport Medium (STM). Positive panel members included CT at 5 and 0.5 fg/reaction and NG at 250 and 50 fg/reaction. A dual-positive panel member combined 5 fg/reaction of CT and 250 fg/reaction of NG, and a negative panel member contained unspiked STM. Panels were tested with the Gen-Probe APTIMA COMBO 2® Assay on 3 prototype Panther instruments weekly over a period of 2 months. The positive and negative agreement with expected results and the inter-run precision were calculated. In addition, panels containing serial dilutions of CT and NG were tested with the APTIMA COMBO 2 Assay on one Panther instrument and one Gen-Probe TIGRIS DTS instrument. Results were compared by plotting the signal generated for each instrument in a scatter plot and calculating the slope using linear regression.

Results: The positive agreement with the expected results was 99% to 100% for all Panther instruments and all runs. Negative agreement was 100%. For CT, within-run precision was ≤10% at 5 fg/reaction and ≤15% at 0.5 fg/reaction. Within-run precision was ≤5% for NG at 250 and 50 fg/reaction. The within-run precision for the dual-positive panel member was ≤5%.

A scatter plot comparing dilution panel results for the Panther and TIGRIS instruments gave a linear regression slope of approximately 0.9. Reactivity rates were 100% for both the TIGRIS and Panther for CT at 0.25 fg/reaction and NG at 12.5 fg/reaction. The Panther processed 120 samples in approximately 5.5 hours.

Conclusion: The newly developed, fully automated Panther molecular diagnostic analyzer provides precise results comparable to the TIGRIS analyzer when running the APTIMA COMBO 2 Assay. In addition to superior assay performance, the Panther offers the optimum throughput, workflow and turn around time that is ideal for the low to mid volume molecular testing laboratory.

External quality assessment for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in clinical biology labs using molecular techniques

B. China*, K. Vernelen, W. Mackay, J.C. Libeer (Brussels, BE; Glasgow, UK)

Objective: Chlamydia trachomatis and Neisseria gonorrhoeae are two of the most prevalent sexually transmitted pathogens. The detection of these two bacteria using molecular techniques was recently included into the nationalcurriculum of the Belgian Health Care Insurance. Therefore, to respect the Belgian legislation and to be reimbursed, the clinical biology labs must participate to an external quality assessment organised by IPH. The aim was to evaluate the proficiency of the labs and of the used techniques.

Methods: External quality samples were provided by QCMD (Glasgow, Scotland) for surveys in 2008. The results were encoded in the QCMD database on their webpage (www.qcmd.org). The response form included the qualitative result (positive, negative or not determined) and all the information about the used technique. Samples were considered as negative, weak positive, positive or strong positive regarding to the level of contamination. A false positive result and a false negative result for a strong positive sample were considered as clinically relevant error and the labs with an incorrect answer received an official claim.

Results: For C. trachomatis, the matrices (urine and swabs) were considered separately. For urine, 96 labs participated with 66.7% of...
good results. The sensitivity and the specificity were 96% and 52% respectively. The percentage of clinically relevant errors was 3.72%. For N gonorrhoeae, a problem of specificity occurred especially with some commercial kits. Therefore, this kind a study allowed to detect proficiency problems either for some of the labs or for some of the used kits.

**P1548** Diagnosis of multiple respiratory pathogens with the Multiplex ligation-dependent probe amplification

R. Jansen*, B.M.W. Diederen, R.A. Guseinova, C.W. Koomen (Haarlem, NL)

**Objectives:** Infections of the respiratory tract are very common in the population and can be caused by a number of different viruses and bacteria. A reliable diagnosis of the pathogen is often cumbersome and time consuming but necessary to prevent superfluous antibiotic treatments, to rationalise isolation of hospital patients and to get insight into the prevalence of respiratory pathogens. The Multiplex ligation-dependent probe amplification (MLPA) of the RespiFinder Plus® kit is a useful tool and its performance on QCMD proficiency panels.

**Methods:** We tested the performance of the MLPA on 420 clinical respiratory samples and on the 2008 QCMD proficiency panels for influenza A/B virus, rhino/corona virus, parainfluenza virus, adenovirus, and respiratory syncytial virus (RSV) human metapneumovirus (hMPV). To mimic mixed infections, the QCMD panels were tested as mixtures. The QCMD-MLPA samples consisted of a mixture of randomly chosen samples of each of the five panels. In total twelve QCMD-MLPA mixtures were analyzed.

**Results:** We analyzed the clinical samples with a maximum turnaround time of three days. The MLPA detected no pathogens in 16% of the samples, a single pathogen in 55% and multiple pathogens in 26% of the samples. In 3% of the samples a non-conclusive result was obtained. RSV was present in 40% of the positive samples, rhinovirus in 25%, coronavirus in 17%, adenovirus in 10%, hMPV in 7%, influenza virus in 6%, and parainfluenza virus in 2%. The bacterial pathogens Mycoplasma pneumoniae and Chlamydia pneumoniae were found in 6 and 1 samples respectively. Bordetella pertussis and Legionella pneumophila were not detected.

In the QCMD-MLPA samples all the virus species and subtypes were identified correctly. The lowest titer of the parainfluenza and influenza panels and a moderate titer of adenovirus type 3 were not detected by MLPA.

A possible drawback of the MLPA is its proneness to cross-contamination, because the test has no closed-tube procedure. No evidence was found for cross-contamination, due to preventive measurements, such as dedicated workspace and pipettes.

**Conclusion:** The MLPA of the RespiFinder Plus® kit is a useful asset and its performance on QCMD proficiency panels is promising.

**P1550** Application of an expanded gold standard and latent class analysis to decide on the strategy for the diagnosis of M. pneumoniae infections

K. Loens*, N. Hens, D. Uris, H. Goossens, M. Aerts, M. Ieven (Antwerp, Diepenbeek, BE)

**Objectives:** Serology and PCR are widely used for diagnosis of *M. pneumoniae* (MP) in respiratory tract infections, but studies comparing the different methods are rare. The aim of this study was to evaluate PCR, NASBA, 2 IgA, and 4 different IgM and IgG EIA assays and the combination of these for the detection of MP in patients with lower respiratory tract infections (LRTI).

**Methods:** 205 paired sera from 224 patients with CAP and 10 paired sera from 20 MP PCR positive patients with LRTI were available. From 29 patients only an acute phase serum sample was available. Four different EIA’s were evaluated: ImmunoWell IgG and IgM EIA (Genbio); Medac IgM, IgA, and IgG EIA (Medac); AniLabsystems IgM and IgG EIA (Biomedical Diagnostics); and Euroimmun IgM, IgA and IgG EIA (Biognost). PCR and NASBA were applied to respiratory specimens.

(1) An expanded gold standard (EGS) and latent class analysis (LCA) were used to calculate the sensitivities and specificities of all individual tests and of the combination of 2 or 3 of these approaches.

**Results:** EGS: The sensitivities of the EISAs ranged from 11−30% for IgM and from 14−22% for IgA in the acute phase serum and from 17−38% for IgM and from 52−62% for IgA in the convalescent serum. IgG seroconversion or a significant rise in titre ranged from 40−69% for IgM and from 17−38% for IgA in the acute phase serum and from 52−62% for IgA in the convalescent serum. IgG seroconversion or a significant rise in titre ranged from 40−69% for IgM and from 52−62% for IgA in the convalescent serum. IgG seroconversion or a significant rise in titre ranged from 40−69% for IgM and from 52−62% for IgA in the convalescent serum. IgG seroconversion or a significant rise in titre ranged from 40−69% for IgM and from 52−62% for IgA in the convalescent serum.

The specificities ranged from 80−99% for IgM and from 84−89% for IgA in both the acute and convalescent phase serum, and from 91−98% for IgG. The MP IgM assay with the best combined values for sensitivity and specificity was the AniLabsystems EIA. The best MP IgG assay was the Medac EIA. (2) The results were validated.
Chlamydia pneumoniae
Evaluation of three commercial serological tests versus
real-time PCR for diagnosis of Mycoplasma pneumoniae
infection in children
K. Papavasileiou*, H. Papavasileiou, A. Voyatzis, S. Chatzipanagiotou
(Athens, GR)

Mycoplasma pneumoniae, (MP) is a common cause of community acquired respiratory tract infections. Laboratory diagnosis of MP infection is usually performed with serological methods. Direct isolation by culturing is time consuming, can be influenced by previously administered empirical antimicrobial therapy and therefore not suitable for clinical practice, while molecular detection methods, such as polymerase chain reaction (PCR) are very sensitive and practical diagnostic tools.

Purpose: The aim of this study was to compare real-time PCR of oropharyngeal swabs with serology, for diagnosis of MP infection, performed by two commercial enzyme-linked immunosorbent assays (ELISA) and one commercial indirect immunofluorescence test (IFT).

Methods: From October 2007 to April 2008, 102 children with clinical signs of lower respiratory tract infection were investigated for MP 1) by real-time PCR (Real time Alert Q-PCR, Nanogen Advanced Diagnostics S.r.l., Corso Torino, Italy), 2) by ELISA IgG-IgM (Hycor, Amsterdam Zuidoost, The Netherlands), 3) by IFT IgG-IgM (Pneumology IgG-IgM, Virceg, Granada, Spain) and 4) by a rapid ELISA-IgM (Immunocardi Mycoplasma, Meridian Bioscience Inc., Cincinnati, Ohio, USA).

Results: MP was detected in 30 children out of 102 (29.4%) with real-time PCR. From these 30 children, 11 (36.66%) were found positive by all three serological tests, 3 (10%) by ELISA IgG-IgM and IgF IgG-IgM, 4 (13.33%) by ELISA IgG-IgM and the rapid ELISA-IgM, 7 (23.33%) only by ELISA IgG-IgM, 3 (10%) only by the rapid ELISA-IgM and 2 (6.66%) only by IFT IgG-IgM. The specificity of all three serological tests as referred to real-time PCR was 100%, while the sensitivity was as follows: 86.66% for ELISA IgG-IgM, 53.33% for IFT IgG-IgM and 60% for the rapid ELISA-IgM.

Conclusions: PCR was superior to serology for the diagnosis of MP infection, while ELISA IgG-IgM proved to be the most sensitive serological method. However, if PCR is practically or technically difficult to performed, the use of two serological tests is warranted.
Use of optical mapping to identify prophages involved in virulence in *Pseudomonas aeruginosa*

T.K. Wagner*, B. Stahl, C.W. Dykes (Madison, US)

**Objectives:** *Pseudomonas aeruginosa* is a Gram-negative bacterium that can be found readily throughout the environment and is a cause of numerous opportunistic infections in humans. Complete full genome sequences have indicated that *P. aeruginosa* strains are highly similar but can differ in genomic islands that can contain virulence genes and genes important in conferring antibiotic resistance. Recent sequencing and animal model data have identified and implicated prophage islands as important factors in the ability of *P. aeruginosa* to cause disease. However, whole genome sequencing to identify these important prophage islands is impractical due to time and cost. In contrast, Optical Mapping can rapidly yield a whole-genome ordered restriction map that can be used to easily detect the prophages. The purpose of this study was to determine whether Optical Mapping could identify prophages that have been shown to be involved in the virulence of *P. aeruginosa*.

**Methods:** Whole-genome Optical Maps were generated from four clinically relevant American Type Culture Collection (ATCC) isolates of *P. aeruginosa* (ATCC 9027, ATCC 17649, ATCC 17651, and ATCC 27317). Optical maps were compared to the recently published Liverpool Epidemic Strain whole-genome sequence that contained three prophages (Prophage 2, Prophage 3, and Prophage 5) shown to increase survival of *P. aeruginosa* during infection. Whole-genome comparative genomics was performed with MapViewer software. MapViewer software was then used to search the ATCC isolate Optical Maps for motifs similar to the motifs of the identified prophages.

**Results:** None of the Optical Maps of the four ATCC isolates contained motifs similar to the three prophages of the Liverpool Epidemic Strain, indicating that the prophages were absent from the isolates. However, sites of phage insertions were identified in all but one ATCC isolate when compared to the prophage-containing reference. Motifs similar to two additional known prophages not yet implicated in virulence were identified in two of the Optical Maps.

**Conclusions:** These data confirm the ability of Optical Mapping to rapidly identify regions in the *P. aeruginosa* genome that contain prophages and the sites of phage insertion. The ability to rapidly generate a whole-genome ordered restriction map to identify prophages can be used to identify more virulent strains and to identify novel genetic elements involved in virulence.

**P1555**

**Fast detection of the V176F mutation of multiresistant *Mycobacterium tuberculosis* isolates using high-resolution melting curve PCR analysis**

A. Pietzka*, A. Stöger, J. Zeinzinger, F. Allerberger, W. Ruppitsch (Vienna, Austria)

**Objective:** In tuberculosis (TB), drug resistance to rifampicin, one of the two most potent first-line drugs, is increasing globally. Resistance to rifampicin is mainly associated with single point mutations in the RNA polymerase gene (rpoB) gene. Several point mutations occur in the 81-bp hot-spot region of cluster I (codons 432 to 458) of the rpoB gene. A frequent mutation outside cluster I associated with high-level resistance to rifampicin is the V176F (GTC176TTC) mutation. To improve the detection of multidrug-resistant (MDR)-TB isolates with wild type cluster I rpoB sequence, we describe a fast and accurate detection method for the V176F mutation based on high-resolution melting curve PCR analysis.

**Methods:** Forty-nine rifampicin resistant and nineteen fully susceptible *Mycobacterium tuberculosis* strains, as determined by conventional drug susceptibility testing, were used to develop a PCR assay to detect the V176F mutation. A 125 bp fragment of the rpoB gene containing the amino acid exchange was amplified for HRM analysis on a LightCycler480 instrument (Roche Diagnostics, Penzberg, Germany).

**Results:** Three of the forty-nine resistant isolates (6%) showed the single nucleotide polymorphism (SNP) class 2 sequence alteration V176F. The remaining forty-six resistant isolates didn’t have any mutation in the 125 bp amplicon as well as the susceptible isolates.

**Conclusions:** Rifampicin resistance is associated with MDR-TB and is therefore a surrogate marker for genotypic drug susceptibility testing. Molecular detection of mutations within cluster I of the rpoB gene is already well known. In addition we describe the feasibility of a new single-step closed-tube screening method for the occurrence of the frequent V176F mutation in the rpoB gene which allows a broad detection of multi drug resistance and which can easily be combined with an assay for mutation testing in cluster I.
availability of MRSA results within two hours of specimen reception facilitates more rapid isolation of MRSA-positive patients.

**Methods:** The study was carried out in a 700-bed tertiary-referral hospital over a 10-week period from October to December 2008. Patients (n = 319) at risk for carriage of MRSA were investigated. Specimens (n = 704) were collected from nasal and groin sites using Copan double swabs in Stuart’s liquid transport medium. One swab was processed by Xpert MRSA; the second was inoculated onto MRSA-Select chromogenic agar (CA) (Bio-Rad) and retained to investigate discrepancies between PCR and culture results. CA plates were incubated for 24 h at 37°C. Discrepancies were investigated by culturing the second swab in salt enrichment with subculture onto blood agar and CA. Xpert MRSA assay results were compared with results of direct and enrichment culture and also on an amended basis where kit-positive culture-negative specimens were considered true positive results if patients had previously been MRSA-positive or had positive samples from another site.

All Xpert MRSA results were reported to the Infection Control Team twice daily but positive results were communicated immediately. Data were collected on the isolation parameters of patients.

**Results:** One-hundred and fourteen specimens were positive by the Xpert MRSA assay and 59 were positive by culture. Sensitivity, specificity, positive and negative predictive values of the Xpert MRSA assay were 95%, 97%, 82% and 99%, respectively, compared with amended results. Five specimens were kit-negative and MRSA culture-positive while 21 specimens were kit-positive and MRSA culture-negative. Six of these samples were confirmed to be false-positives. Seventy-six patients (24%; 76/319) were positive by PCR. On assessment of the impact of these rapid results: six were discharged from hospital and 19 were already isolated/cohorted before results were reported while 51 were isolated/cohorted following the positive result. Seventy percent (36/51) were isolated within 8 hours of specimen result which is up to 48 hours earlier than occurs with routine culture results.

**Conclusions:** Rapid screening using the Xpert MRSA assay greatly facilitated earlier isolation of MRSA-positive patients and combined with good diagnostic accuracy can contribute to improved MRSA control.

**Table:** Performance of three commercial assays for rapid detection of MRSA

<table>
<thead>
<tr>
<th>Assay</th>
<th>Mean sensitivity (95% CI) at MRSA concentration</th>
<th>Mean specificity (95% CI)</th>
<th>Minimum limit of detection (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneOhm™ MRSA</td>
<td>97.8% (96.6–99.4)†</td>
<td>98.5% (97.9–99.2)†</td>
<td>4000†</td>
</tr>
<tr>
<td>GeneXpert™ MRSA</td>
<td>98.4% (98.1–98.7)</td>
<td>99.8% (99.2–100)</td>
<td>4000*</td>
</tr>
<tr>
<td>Baclite™ MRSA (5M, USA)</td>
<td>95.3% (95.7–95.9)</td>
<td>99.5% (99.5–99.7)</td>
<td>140‡</td>
</tr>
</tbody>
</table>

*No LoD determined. Only samples negative at the lower concentration (n = 2) were tested at this concentration.

**P1559** Evaluation of commercially available molecular and culture-based assays for rapid detection of methicillin-resistant *Staphylococcus aureus*

W Sabiti, J Cortinhas Abrahantes, C Lammens, G Molenberghs, M Acers, H Goossens, S Malhotra-Kumar on behalf of the MOSAR WP2 Study Group

**Background:** The need for rapid methods to accurately detect methicillin-resistant *Staphylococcus aureus* (MRSA) is widely acknowledged. We assessed 3 commercial assays – 2 molecular, GeneOhm (BD Diagnostics) and GeneXpert (Cepheid) – and 1 culture-based, Baclite (3M) – for their ability to correctly identify MRSA utilising well-characterised isolates, either pure or in mixtures, at varying concentrations.

**Methods:** Fifty-two isolates (27 MRSA of which 9 were animal strains; 25 non-MRSA of which 8 were MRCoNS, 5 MRSA, and 12 enterococci, Enterobacteriaceae and Acinetobacter spp.), and 21 mixtures of these isolates were tested on the three assays following manufacturer’s recommendations. As pure strains, non-MRSA were tested at 1 dilution (10³ CFU/ml for GeneOhm and GeneXpert, and 10⁴ CFU/ml for Baclite according to manufacturer’s instructions), while serial dilutions (10³ to 10⁶ CFU/ml) of the 27 MRSA strains were tested to determine the limit of detection (LoD) of each assay. Mixtures were made using isolates at varying concentrations according to CLSI guidelines. Moreover, 10 μl of each sample was also simultaneously spiral-plated on blood agar with 6 μg/ml cefoxitin and in case of MRSA positive samples, colony counts were made after overnight incubation. Mean sensitivity and specificity and confidence intervals (CIs) were estimated for each assay by logistic regression model using a penalised likelihood approach.

**Results:** GeneOhm showed the highest sensitivities for both isolates and mixtures at lower (10³ CFU/ml) concentrations (Table).

**Objective:** Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasing problem in nosocomial infection. Rapid detection of MRSA-colonised patients is a prerequisite to limit spread of the organism in hospitals. Several molecular assays to quickly identify MRSA are available. The performance of such assays depends on many factors among which sensitivity and specificity of the assay. Besides, the incidence and type of MRSA strains that are locally present (foreign influence, presence of animal farms) influence positive and negative predictive values.

**Methods:** We evaluated the performance of four molecular methods on an ABI 7500 platform (BD GeneOhm MRSA real-time PCR assay, Hain GenoType MRSA direct and two in-house assays) using samples from 226 patients. In house PCR-1 was based on the detection of a *S. aureus* specific gene together with the MecA gene, in house PCR-2 was an MRSA direct PCR adapted from Huletsky. A minimum of two samples (each containing swabs from nose, throat and perineum) were analysed per patient. Before amplification, samples were enriched by overnight incubation in Tryptose Phosphate Broth with aztreonam. After overnight incubation CHROMagar MRSA and blood agar plates were inoculated. The outcome of the cultures after 2 days was considered gold standard.

**Results:** Twenty-three of the 226 patients carried MRSA. The sensitivities of the BD GeneOhm, Hain MRSA direct and in house PCR-1 and -2 were 95.7%, 95.6%, 69.9% and 87% respectively. The positive predictive values were 62.9%, 59.5%, 25%, and 33.3% and the negative predictive values were 99.5%, 99.5%, 95.3% and 98.2% respectively. The MRSA that were missed after overnight culture were detected as pure cultures. One MRSA strain remained negative in both the BD and Hain test, none were negative in PCR-1 and PCR-2.

**Conclusions:** We conclude that in our geographical region both the BD GeneOhm MRSA test and the Hain MRSA direct test displayed excellent
sensitivities and negative predictive values to detect MRSA in overnight cultures. Considering the relative small turnaround time of the BD test, after overnight incubation (3 hr BD versus 6 hr for the Hain test) and the advantage of amplification detection in a closed system, the BD test offers us a welcome tool for quick MRSA screening.

**P1560 Detection of methicillin-resistant *Staphylococcus aureus* in skin/soft tissue samples using a commercial PCR method**

B. Puch, E. Ubeda, J.L. Garcia-Lopez, M. Marin, C. Castro, F. Lucena, E. Martin-Mazuelos

**Objective:** Prevalence of community-acquired methicillin resistant *S. aureus* (MRSA) has increased in the last decade, becoming one of the most frequent causes of skin/soft tissue infections (SSTI). In order to avoid inadequate empiric antibiotic therapy and to take isolation measures, early detection of MRSA is of major importance. Our aim was to evaluate a commercial PCR method for the detection of MSSA/MRSA on SSTI samples compared to conventional culture methods.

**Methods:** A prospective analysis was carried out in patients with SSTI attending the ER of our hospital and requiring hospitalisation during 2008. Three samples were tested per patient. One nasal and one SSTI swab were processed following conventional culture on blood agar, MacConkey agar, chocolate agar and chromogenic MRSA agar plates. Another SSTI swab was used for PCR analysis using the XpertTM MRSA/SA SSTI Assay (Cepheid Innovation) on the GeneXpert platform following manufacturer’s instructions.

**Results:** 139 patients were included, with a mean age of 67.4 years (± 15.67). Within the risk factors for the presence of MRSA, the most common were chronic wounds (47.5%), diabetes (44.6%), hospitalisation (43.2%), antibiotic therapy (28.9%), institutionalisation (7.2%) and/or previous detection of MRSA (4.3%). Cellulitis was observed in 47.5% of the patients, diabetic foot ulcer in 29.5% and pressure ulcer in 20.1%. In total 148 samples were analyzed. In 9 cases, PCR was invalidated and in 7 conventional culture was not carried out, of which 6 were negative and one was MSSA by PCR. Of the remaining samples, 72 were negative and 32 positive either to MSSA (25 samples) or MRSA (7 samples) by both methods. False negative results were observed in 25 samples (23 cultures and 2 PCRs), whereas discordances in methicillin resistance was observed in 3 samples, all MSSA by PCR. Time employed for conventional culture varies from 24 to 72 hours while PCR is performed in 1 hour, approximately. Compared to conventional culture, sensitivity of PCR was 100%, specificity was 99%, precision rate was 0.90 and NPV was 1.

**Conclusions:** 1. Community-acquired MRSA is present in 7.6% of SSTI in our region. 2. This commercial PCR method is rapid, sensitive and accurate in the detection of both MSSA and MRSA in SSTI samples, with high precision rate and NPV. 3. The differences in the detection of resistance to methicillin are probably due to the presence of other mechanisms, rather than the mecA gene.

**P1561 Performance of Becton Dickinson GeneOhm methicillin-resistant *Staphylococcus aureus* PCR assay in different spa- and PFGE-types of MRSA**

J. van der Velden, A. Heumassej, M. Hermans, T. Trienekens

**Objective:** Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasing problem in nosocomial infection. Rapid detection of MRSA-colonised patients is a prerequisite to limit spread of the organism in hospitals. Several molecular assays to quickly identify MRSA are available. Beside assay design, the incidence and types of MRSA strain that are locally present (foreign influence, presence of animal farms) influence the performance of such assays. We evaluated the ability to detect different *Staphylococcal* Protein A (spa) and pulsed-field gel electrophoresis (PFGE) types of MRSA with BD GeneOhm MRSA real-time PCR assay on an ABI 7500 platform.

**Methods:** In total seventy-nine MRSA strains were tested. Thirty-one strains were collected since autumn 2005 from the region of Venlo and selected to include as many spa-types as possible in the evaluation. To collect more different types, also strains from the region of Den Bosch were included. From those strains mostly PFGE typing was available (typing was done at the RIVM, Biltoven, the Netherlands), because before 2007 MRSA strains were not routinely spa-typed in the Netherlands. A disadvantage of PFGE-typing is that data cannot be compared between different laboratories. Spa-typing, in contrast, is sequence based and its results are valid worldwide. Hence, if both typing results were available only the spa type was considered in our evaluation. From some spa-or PFGE-types more than one MRSA strain was collected.

**Results:** Thirty-six different PFGE types were collected from which 4 strains with 4 different PFGE-types were not detected. From the 29 different spa types 2 MRSA strains with 2 different spa-types were not detected. In the region of North-Limburg the predominating spa types were 1011 and 1108. These are animal farm related types. The other spa-types obtained from this region were isolated only once or twice.

**Conclusion:** We conclude that the BD GeneOhm MRSA test missed several MRSA strains. However in our region, with a specific selection of MRSA types, the BD GeneOhm test performed well for MRSA screening purposes.
Direct detection of methicillin-resistant *Staphylococcus aureus* in broth-enriched clinical samples using a real-time multiplex PCR

B. Söderquist*, L. Stark, J. Zimmermann, M. Neander, C. Berglund, S. Melin, A. Matussek, P. Mölling (Orebro, Jonkoping, SE)

**Objectives:** A rapid, reliable, and sensitive method for methicillin-resistant *Staphylococcus aureus* (MRSA) screening is warranted in a low-endemic area such as Scandinavia. A method for excluding the presence of MRSA in clinical samples using an MRSA-selective enrichment broth incubated over night followed by PCR of the nuc gene is widely used. However, a method for direct detection of MRSA in clinical samples would further facilitate the infection control management of patients and hospital staff.

**Methods:** A real-time multiplex PCR for direct detection of MRSA using a *S. aureus* orfX-specific primer and a set of SCCmec-specific primers was developed on a LightCycler PCR system (Roche Diagnostics). The study included i) broth enriched clinical samples pooled from patients; DNA prepared manually (n=1328), manually compared with an automated Tecan Minipur 75 with BUGS’n BEADS® MRSA kit (NorDiag ASA) (n=130), and the latter compared with a fully automated (from primary tubes) Xiril with BUGS’n BEADS® STI-Fast (NorDiag ASA) (n=308), ii) MRSA strains of different spa types and SCCmec types (n=171), iii) methicillin-sensitive *S. aureus* (MSSA) isolates (n=95) and iv) various coagulase-negative staphylococcal (CoNS) isolates (n=33). The multiplex PCR was compared with the nuc screening PCR using the broth enriched clinical samples.

**Results:** The multiplex PCR detected all MRSA strains as well as all broth enriched clinical samples with MRSA (n=64) and none of the CoNS were positive. MSSA strains were positive in 4/95 (4%). One to three percent of consecutive broth enriched clinical samples were false positive in the multiplex PCR and subsequently subcultured compared with 12% of samples analysed with nuc screening PCR. The automated DNA preparation on the Tecan Minipur 75 was better compared with the manual preparation resulting in lower crossing point values and the fully automated Xiril was even more efficient.

**Conclusion:** The developed real-time multiplex PCR using a *S. aureus* orfX-specific primer and a set of SCCmec-specific primers was sensitive and detected all heterogenic and diverse MRSA strains present in our low endemic area. Four percent of MSSA was false positive. However, the percentage of clinical samples demanding further subculturing decreased with about 10% using the multiplex PCR. The automated DNA preparation facilitates handling of a large amount of samples such is the case in MRSA outbreaks.

Multiple-locus variable-number tandem repeat analysis in a setting of polyclonal endemicity of methicillin-resistant *Staphylococcus aureus*

B. Rico-Pláza*, E. Pérez-Roth, S. Méndez-Ávarez (La Laguna, Santa Cruz de Tenerife, ES)

**Objectives:** The methicillin-resistant *Staphylococcus aureus* (MRSA) population in the Hospital Universitario Nuestra Señora de Candelaria (HUNC) over a 5-year period was marked by shifts in the circulation of pandemic clones. The aim of the study was to investigate if a MLVA (Multi-Locus Variable number tandem repeat Analysis) assay could predict MRSA clones previously identified by PFGE and supported by MLST and SCCmec typing. Also to establish possible criteria of clustering MLVA patterns and to check concordance levels between the results produced by MLVA and other typing methods. This study expects to validate the MLVA, thus allowing us to introduce MLVA as a routine typing method in the HUNC.

**Methods:** Over a five year period, 296 well-characterised isolates belonging mainly to 5 major pandemic lineages of MRSA were tested by MLVA. MLVA was carried out by a multiplex PCR in which clfA, clfB, sdrCDE, spaA, and spa locus were amplified. Banding patterns were analyzed both visually and with InfoQuest software. We also quantitatively assess the congruence between typing methods by using Adjusted Rand index (AR) and Wallace's coefficient (W).

**Results:** All the isolates were typeable by MLVA, and the results were repetitive. PFGE was able to divide the 296 isolates into 20 PFGE types (>six bands different) and 41 subtypes (one to six bands different). The 296 isolates were classified in 35 SCCmec types (one different band cut off), 17 types (80% cut off) and, 11 types (70% cut off). The discrimination power of PFGE was higher (D=75.14[70.28–79.99]) than for MLVA (D=71.33[66.14–76.52]). The higher congruence between PFGE and MLVA was found applying the 80% cut off criteria for MLVA, with a value of AR=0.86. Thus, two strains with the same MLVA type have 83.63% chance of having the same PFGE type. This value is improved to 84.46% with the one different band criteria. When comparing partitions obtained by international nomenclature, ST-SCCmec, the higher congruence was achieved with MLVA 80% cut off, with AR=0.98. Applying this cut off, two strains with the same MLVA type have 99.09% chance of having the same PFGE type. With the one different band cut off criteria, this probability rises to 99.33%. MLVA typing has a very good predictive power over the clonal relationships defined by PFGE and ST-SCCmec typing.

**Conclusion:** Our results suggest that MLVA may be reliable for the identification of endemic MRSA clones circulating in the HUNC.

Comparisons of healthcare-associated and community-associated MRSA strains using SCCmec EVIGENE and routine susceptibility testing

A.K.I. Rasmussen, A. Mortensen, D. Hong*, H.K. Johansen (Vedbaek, Copenhagen, DK)

**Background:** Healthcare-associated (HA) methicillin-resistant *Staphylococcus aureus* (MRSA) possess multiple antimicrobial resistance determinants and are typically multidrug resistant with a SCCmec type I, II or III. In contrast, SCCmec IV community-associated CA-MRSA strains are generally susceptible to non-β-lactam antibiotics including clindamycin and glycopeptides.

**Methods:** In the current study we compared the antimicrobial patterns between CA-MRSA and HA-MRSA in Danish strains. Identification of HA-MRSA and CA-MRSA were done with SCCmec EVIGENE (AdvanDx) a signal-amplifying, sandwich hybridisation assay which provides results within 3 hrs. 67 clinical MRSA isolates from Denmark (Rigshospitalet) were tested by SCCmec EVIGENE for the presence of each of the five SCCmec types, mecA (confering resistance to methicillin), nuc (S. aureus specific nuclease gene) and PVL (virulence genes for Panton-Valentine leukocidin). Antimicrobial susceptibility patterns were acquired by agar disk diffusion using Neosensitabs (Rosco).

**Results:** Thirteen % (9/67) of the 67 MRSA positive isolates were identified as HA-MRSA strains by the presence of SCCmec type I (1), II (7) or III (1) and 81% (54/67) were identified as CA-MRSA strains by the presence of SCCmec type IV without PVL 40% (27/67), type IV with PVL 34% (23/67) and 6% (4/67) with multiple SCCmec types (1&4 or 1&4&5 or 4&5). All multi-types were identified as CA-MRSA as the type I marker (plp) has in few cases been found in type IV. Six % (4/67) were negative for all five SCCmec types and therefore unidentified. All tested MRSA isolates were resistant to penicillin and cefuroxime. When comparing antimicrobial resistance patterns between HA-MRSA and CA-MRSA we found that HA-MRSA have a higher prevalence of resistance to azithromycin & clindamycin than CA-MRSA (89% (8/9) vs. 36% (19/53)), and to gentamicin (33% (3/9) for HA-MRSA vs. 2% (1/53) for CA-MRSA). Furthermore, CA-MRSA negative for PVL have a higher prevalence of resistance to azithromycin & clindamycin compared to CA-MRSA positive for PVL (66% (19/29) vs.13% (3/24)).

**Conclusions:** SCCmec EVIGENE is an easy diagnostic tools to differentiate HA-MRSA (type I, II, III) and CA-MRSA (IV, V) strains. Comparisons of Danish HA-MRSA vs. CA-MRSA isolates showed that HA-MRSA were more likely to be resistant to azithromycin,
Molecular typing of enterotoxigenic strains of *Staphylococcus aureus* isolated from different foods of animal origin

G. La Salandra*, G. Normanno, E. Crisetti, L. Serreccchia, T. Onni, M. Fiori, V. Lorusso, S. Toha (Foggia, Bari, Sassari, IT)

**Objectives:** Staphylococcal food poisoning (SFP) remains one of the most important cause of foodborne diseases in industrialised countries. Because of the great genetic variability of *Staphylococcus aureus*, its ability to synthesise an extensive number of staphylococcal enterotoxins (SEs), and its large host spectrum, results from epidemiological investigations and risk analyses were often difficult to assess. The aim of this work was to genotypically characterise 159 enterotoxigenic strains of *S. aureus* isolated from food of animal origin (milk, cheese, meat and fish products) and pastry products, collected in Southern Italy during 2007–2008, in order to detect the SEs coding genes, and to obtain a genetic fingerprinting of the strains.

**Methods:** Genomic DNA extracted from strains of *S. aureus* was analysed for the detection of 12 SE genes (sea, seb, sec, sed, see, seg, seh, sei, sej, sem, sen, seo) using three multiplex-PCR assay. The enterotoxigenic strains were tested by pulsed field gel electrophoresis (PFGE) after Smal restriction treatment. DNA restriction bands were analysed using the Gel Compar II software (Applied Maths). Dendrograms were obtained using the unweighted pair group method with arithmetic mean (UPGMA) and Dice’s coefficient.

**Results:** The results showed a prevalence of classical SEs encoding genes (sea, seb, sec, sed and see) compared with the “new” SEs encoded genes; the new SEs encoding genes are generally associated with the classical ones. Overall out of 159 analysed strains, 59 (37.1%) *S. aureus* isolates were found to be positive for only one se gene, 52 (32.7%) for two, 30 (18.9%) for three, 5 (3.1%) for four, 10 (6.3%) for five and 3 (1.9%) for six se genes. All the strains were typable by PFGE and showed a great genetic heterogeneity especially among the bovine food isolates.

**Conclusion:** The present work might help to trace an epidemiological map of different enterotoxigenic strains and to investigate the possible correlations between genotype and presence of enterotoxin genes. The results obtained confirm the high frequency of the SEs encoded genes among *S. aureus* and the potential risk for the consumer, if good hygiene practices are not observed. In terms of risk analysis, it could be important to better understand the source and the significance of the presence in foods of *S. aureus* strains carrying multiple se genes and whether these strains are more likely to synthesise SEs in foods than the strains carrying only one se gene.

Molecular epidemiology of MRSA

**P1567** Molecular detection of *meca* and *pvl* genes in clinical setting


**Objectives:** The aim of the study was to investigate the presence of *pvl* and *meca* genes of *S. aureus* strains isolated from clinical samples in our laboratory.

**Materials and Methods:** From January to October 2008, 217 *S. aureus* strains were isolated from different clinical samples, corresponding to 7.4% (217/2945) of positive cultures. In particular, 35 (8.3%) *S. aureus* strains were isolated from blood cultures, 48 (18.9%) from pus or wound infections, 84 (9.4%) from respiratory samples and 50 (3.6%) from various other samples. All strains were identified using Vitek2 automated system (Biomerieux, France). Antibiotic susceptibility testing was performed by Vitek2 and manual Kirby-Bauer method (EUCAST guidelines). All oxacillin resistant strains were further tested by PCR for the presence of *meCA* and *pvl* genes (Genotype *Staphylococcus*, Hain Lifescience).

**Results:** From a total of 217 *S. aureus* strains, 129 were identified as MRSA (59.4%) by Vitek2. All MRSA strains carried *meca* gene. 18/35 (51.4%) of *S. aureus* strains isolated from blood cultures were MRSA, whereas 2 of them (11.1%) carried the *pvl* gene. In addition, 27/48 (56.3%) of *S. aureus* strains isolated from pus were MRSA. The *pvl* gene was found in 9 out of 15 (60%) tested by PCR. In respiratory samples, 51 MRSA strains were identified (51/84, 60.7%), and among these, 4 (7.8%) carried the *pvl* gene.

**Conclusions:** In the present study, virulent, *pvl* positive MRSA strains were isolated from various clinical specimens. In respiratory samples, MRSA strains were frequently isolated indicating either a carrier’s status or active infection. Confirming previous reports, all strains carrying the *pvl* gene, were also carrying the *meca* gene. The highest rate of *pvl* (+) MRSA strains was isolated from pus samples. Molecular methods have revolutionised clinical practice in the medical microbiology laboratory by providing a suitable tool for the rapid and sensitive detection of bacterial virulence and resistance to antibiotics.

**P1568** Vancomycin-intermediate *Staphylococcus aureus* from bloodstream infections in Italy

M. Monaco*, A. Sanchini, F. D’Ambrosio, A. Fantosti (Rome, IT)

**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of morbidity and mortality in hospital settings. Vancomycin still represents the drug of choice for treating MRSA infections, although reduced susceptibility or resistance to vancomycin has emerged. The aim of this study was to examine invasive *S. aureus* strains in order to investigate the presence of vancomycin-intermediate *Staphylococcus aureus* (VISA) or hetero (h)VISA and to characterise them using molecular typing methods.

**Methods:** In the period 1 September 2006 – 28 February 2007 150 *Staphylococcus aureus* strains, mostly from bloodstream infections, were obtained from 19 hospital laboratories distributed all over the country, within the joint EARSS/SeqNet.org initiative. Eighty-four were MSSA and 66 MRSA. Susceptibility to vancomycin was assayed by E-test using CLSI breakpoints. VISA/hVISA screening was performed according to E-test protocol. Vancomycin population analysis profile (PAP) was carried out on selected strains. The agr group and the staphylococcal chromosomal cassette (SCCmec) were determined by PCR. The repeat region of the *S. aureus* protein A (spa) gene was sequenced and analysed by the Ridom Staph Type software. MLST was also performed.

**Results:** Vancomycin susceptibility for MSSA was: MIC50 = MIC90 = 1.5 mg/L; for MRSA: MIC50 = 1.5 mg/L, MIC90 = 2 mg/L. Among MSSA no strains were VISA or hVISA. Among MRSA 3 strains were VISA (MIC = 3 mg/L) and 10 isolates hVISA. PAP confirmed heteroresistant subpopulations in the presence of 4 mg/L of vancomycin. By spa typing, MSSA showed high heterogeneity among while MRSA three main groups were identified: 041(23 isolates), 008(19 isolates) and 001(9 isolates). Out of 3 VISA, 2 were assigned to spa type 041; of 10 hVISA, 9 were assigned to spa type 041. All the isolates assigned to spa type 041 harboured SCCmec type I and agr 2 that is mostly associated with reduced susceptibility to vancomycin. By MLST, all VISA and h-VISA isolates belonged to clonal complex (CC)35.

**Conclusions:** VISA and h-VISA were detected only among MRSA. The majority of VISA and h-VISA were assigned to spa type 041 which is the most frequent spa type among MRSA from bloodstream infections in Italy.

**P1569** Skin lesion caused by ST398 and ST1 methicillin-resistant *Staphylococcus aureus* in a young girl in Spain and MRSA nasal colonisation study in their family members

C. Aspíroz, C. Lozano, A. Vindel, J.J. Lasarte, M. Zarazaga, C. Torres* (Zaragoza, Logrono, Madrid, ES)

A 12 years old Ecuadorian girl, living close to a pig farm where her father was working, presented an skin lesion on her chin (June 2008), firstly
assumed as a case of Tinea but dermatophytes could not be recovered, and this diagnosis was later discarded. Two types of MRSA were the unique organisms that could be recovered from the lesion and they were characterised by MLST, SCCmec and spa typing and were identified as ST398-SCCmecVI-011 and ST1-SCCmecII-t127, being both isolates negative for the Panton Valentine leucokidin. The ST398 strain showed resistance to penicillin, oxacillin, tetracycline, erthyromycin, clindamycin and tetracythromycin and contained the mecA, tetK, ermA, and ermC genes. The ST1 strain showed the above mentioned resistances and genes, and in addition included gentamicin, tobramycin, kanamycin, ciprofloxacin, levofloxacin and trimethoprim-sulphamethoxazole resistances and msrA, emrB, tetL, ant(4")4", ahp(2")-aac(6") genes, as well as a Ser84Leu amino acid change in GyrA and a Ser80Phe change in ParC protein. The skin lesion was resolved after topical treatment with mupirocin during ten days. An epidemiological study was started in order to know the patient's family members, using nasal swabs that were streaked in ORSAB medium (Oxoid) for MRSA recovery and obtained isolates where characterised by MLST, SCCmec and spa typing. The following family members turned out to be colonised by MRSA: patient girl (ST398-SCCmecVI-t108), father (ST398-SCCmecVI-t108), mother (ST398-SCCmecVI-t108 and ST1-SCCmecII-t127), and brother (ST398-SCCmecVI-t011). Most of ST398 nasal isolates presented the phenotype of resistance that included l-lactams, macrolides, clindamycine, tetracycline and tetracycline. The nasal ST1-t127 isolate recovered from the mother showed the same phenotype and genotype of resistance that ST1-t127 strain recovered in the skin lesion of the girl. All isolates were negative for TSST, ETA-a and ETA-b toxins.

Conclusion: The first case of an skin lesion associated with ST398-t011 and ST1-t127 MRSA isolates is reported in Spain in the daughter of a pig farmer. Of special interest is the multiresistant-phenotype showed by the ST1-t127 MRSA clinical isolate. Nasal colonisation by different ST398 genetic lineages of MRSA seems to be frequent in persons that live close to pig farms. Dissemination of ST398-MRSA in humans, associated with animals, is an emerging problem that should be tracked in the future.

**Epidemiology of methicillin-resistant *Staphylococcus aureus* in the dermatology department of a university hospital, Casablanca, Morocco**


**Objectives:** Methicillin resistance *Staphylococcus aureus* (MRSA) is a common pathogen amongst nosocomial infections in Morocco. At the University Hospital Ibn Rochd most of the isolated *Staphylococcus aureus* strains came from samples of patients hospitalised in the Dermatology Department. In order to better understand the epidemiology of MRSA in dermatology, we determined the antibiotic susceptibility of the isolated strains and their genetic profiles.

**Methods:** A prospective study was undertaken between January and March 2007: 13 MRSA were isolated during this period in Dermatology Department. The strains were characterised by using susceptibilities to 16 antibiotics using agar disk diffusion method according to CLSI guidelines. The methicillin resistance was confirmed by the presence of gene mecA by PCR. Genetic typing of the agr system and the staphylococcal cassette chromosome mecA (SCCmecA) were realised by multiplex PCR, and the sequence type of these strains was determined by MLST.

**Results:** The MRSA prevalence in Dermatology Department raised 30.3% while the general MRSA prevalence in the hospital is 19.1%. The MRSA strains were resistant to Tetracyclin, Pefloxacine, Trimethoprim-sulphamethoxazole, Fucidic acid and Erythromycin; 11 strains were also resistant to Rifampin and 12 to Kanamycin, Gentamycin and Tobramycin. The genetic typing demonstrated that all strains are agr1, SCCmec III and belong to the Sequence Type 241, (ST241) showing the same profile than Hungarian clone.

**Conclusions:** The high prevalence of MRSA in Dermatology Department is related to the spread of the multidrug resistant Hungarian clone, thus limiting the choice of effective antibiotics for MRSA infection treatment.

**Present prevalence of Panton-Valentine leukocidine-positive methicillin-resistant and susceptible *Staphylococcus aureus* in Spain**

E. Cercenado*, M. Martin, R. Insa, O. Cuevas, A. Vindel, E. Bouza (Madrid, Majadahonda, ES)

**Objectives:** At present, the prevalence of Panton-Valentine leukocidin-positive (PVL+) community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Spain is low, however two nationwide surveys have demonstrated that the prevalence of PVL is increasing among methicillin-susceptible *S. aureus* (MSSA) (from 1.6% in 2002 to 18% in 2006). In this study we determine the present prevalence of PVL+ MSSA and PVL+ MRSA in Spain.

**Methods:** From November 2007 to January 2008 we investigated the presence of the PVL in all *S. aureus* recovered in our laboratory; in addition, from February to November 2008 the presence of PVL was investigated in all MRSA isolates. The PVL genes were detected by PCR. SCCmec types were determined using a multiplex PCR. PGFE and MLST typing of the PVL+ MRSA was performed as described.

**Results:** Over the first period of the study, a total of 454 *S. aureus* were isolated in our laboratory (272 MSSA and 182 MRSA). Of the MSSA 5.5% (n = 15) were PVL+, and corresponded to 11 patients (6 children, 5 adults); of the MRSA 1.1% (n = 2) were PVL+. MSSA PVL+ isolates were from skin and soft-tissue (SST) (n = 9), blood (n = 3), bone (n = 2), and peritoneal fluid (n = 1). Over the second period a total of 691 MRSA isolates were recovered and 9 were PVL+ (1.3%). All PVL+ isolates were community-acquired. Overall, PVL+ MRSA were from SST (n = 10; 8 adults, 2 children), and blood (n = 1; child; resistant to erthyromycin and clindamycin). Five PVL+ MRSA isolates were from Ecuadorian patients, 5 from Spanish patients and 1 from an African patient. All belonged to ST8-SCCmecIV. None of the isolates belonged to the USA300 clone. All patients recovered after adequate treatment.

**Conclusions:** At present, infections due to PVL+ MRSA are not a cause for concern in our area. However surveillance of PVL+ MSSA should be considered, since the higher prevalence of the PVL among our MSSA isolates could establish a background for the emergence of PVL+ MRSA.

**Prevalence of genes encoding pyrogenic toxin superantigens, the Panton-Valentine leukocidin and exfoliative toxins in *Staphylococcus aureus* isolated from furunculosis – association with agr group**

H. Masiah*, S. Holtfreter, J. Kolata, B. Brüker, S. Giedrys-Kalemba (Szczecin, PL, Greifswald, DE)

*Staphylococcus aureus* is an important skin and soft tissues community-acquired infections pathogen, that produces many extracellular virulence factors. There are Panton-Valentine leukocidin ( lukS-lukF genes), pyrogenic toxins (eta, etb, etd), pyrogenic toxin superantigens (SAg) comprising TSST-1 (tsl) and the staphylococcal enterotoxins (sea-r, seu).

The genes are located on mobile genetic elements. The gene expression of some factors is controlled by a complex network of global regulators, including agr (accessory gene regulator, groups I-IV).

The aim of this study was to investigate the relationship between toxin gene profile of *S. aureus* isolates from furunculosis and agr group.

**Methods:** 75 *S. aureus* strains were collected between 2002 and 2008 from West Pomeranian region (Poland) patients with furunculosis. For agr typing and for the detection of 19 SAg genes (sea, seb, sec, sed, see, seg, she, sei, sej, sek, sel, sem, sen, seo, sep, seq, ser, seu, tst) and exfoliative toxins (eta, etd) we performed a set of six multiplex-PCRs. The prevalence of lukS-lukF and mecA gene was determined by PCR.

**Results:** Most of the isolates (56%) belonged to the agr IV group (all carried the enterotoxin gene cluster: seg, sei, sem, sen, seo and most additionally seb), 28% – agr I (most carried seg, sei, sem, seu, seo, single seg, sei, sem, sen, additionally seb, sel, seh, sek, seq or none
Characterisation of Panton-Valentine leukocidin positive methicillin-resistant Staphylococcus aureus in Italy

S. Stefani*, M. Monaco, F. Campanile, V. Cusfo, A. Sanchini, P. Marone, A. Pantusti (Catania; Rome; Pavia, II)

Objectives: Recent reports show a dramatic increase worldwide of infections caused by community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA). These strains, by definition, infect patients with no risk factors for acquiring nosocomial MRSA strains, and are also well characterised by genetic markers such as the presence of SCCmec type IV, V or VII and the presence of the Panton-Valentine leukocidin (PVL). The aim of this study was to characterise a collection of PVL-positive CA-MRSA strains isolated in Italy.

Methods: 15 PVL-positive CA-MRSA strains were collected from 2005 to 2008 in Italy from severe infections: 6 from skin and soft tissue infections, 1 from a brain abscess, 6 from necrotising pneumonia and 2 from disseminated infections. The phenotypic and genotypic characteristics of the strains were determined including antibiotic susceptibility, SCCmec and agr types, protein A (spa) types, multilocus sequence types (MLST) and toxin profiles.

Results: Most of the PVL-positive CA-MRSA strains were susceptible to all non-lactam antibiotics with few exceptions: resistance to tetracyclines (3 isolates), to gentamicin (1 isolate) and to fluoroquinolones (1 isolate). Thirteen isolates harboured SCCmec type IV and 1 isolate type V. The agr types were: 1 (7 strains), 2 (3 strains) and 3 (4 strains). Six different spa types were assigned: 008 (5), 044 (1), 1319 (1), 1755 (1), 2453 (1), 2526 (1). The strains belonged to 5 different STs, namely ST5, ST8, ST30, ST80 and ST88. With the exception of a strain belonging to ST8 that carried superantigenic toxin genes, all other isolates encoded other pore-forming leukotoxins, such as alpha, delta, gamma-haemolysins and lukE; in addition they harboured the genes for a serin protease like-B, pore-forming leukotoxins, such as alpha, delta, gamma-haemolysins and lukE; in addition they harboured the genes for a serin protease like-B, and various adhesins such as fibronectin A, sdrC and sdrE, adhesion factors for fibrinogen, and both polysaccharide intercellular accumulation protein (icaA) and initial attachment adhesion (aft).

Conclusion: We documented the isolation of PVL-CA-MRSA clones in Italy, belonging to different lineages. All these clones possessed virulence determinants that are more common in invasive isolates, and together with the ability of some strains to produce biofilm, could contribute independently to virulence.

Molecular epidemiology of methicillin-resistant Staphylococcus aureus in a northern Italian hospital

C. Purlato*, P. Cavallero, L. Fossati, T. Alice, R. Serra (Turin, IT)

Objectives: We conducted a one-year study on the epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) in our hospital, San Giovanni Battista of Turin (Italy), from August 2007 to August 2008. The aim was to identify one or more MRSA clones within circulating in 58 wards, including Intensive Care Units (ICUs), surgical and specialist surgical units, general and specialist medicines, transplant organ units, using molecular epidemiological methods.

Methods: 225 MRSA isolates were derived from clinical samples of patients with active infections like surgical wound infections (22%), urine (16%), blood (15%), bronchoaspirate (13%), endovascular catheter (8%), wound infections (8%), bronchoalveolar lavage (5%), tracheal aspirate (4%), cerebrospinal fluid (3%), peritoneal fluid (2%), drainage (1%), biopsy (1%), bile (1%), pericardial fluid (1%), transplant organ preservation fluid (0.5%) and peritoneal exhaust (0.5%). All isolates were analysed by PFGE, rep-PCR, SCCmec typing, VISA typing and spa-typing.

Results: The isolates were classified into following SCCmec types: 48 SCCmec type I (21.3%), 106 SCCmec type II (47.1%), 2 SCCmec type III (0.8%), 66 SCCmec type IV (29.3%) and 3 SCCmec type V (1.3%). Of the SCCmec type IV, only 2 were positive for VISA (0.9%). Overall, 20 spa types were detected, with t242, 008 and 001 being most prevalent (44.9%, 23.5% and 21.3%, respectively). PFGE and rep-PCR showed specific profiles that reflect the molecular features of strains.

Conclusion: using molecular typing and sequence-based methods we were able to identify the presence of three MRSA clones within the hospital. The most common strain is t242 with SCCmec type II. In addition to the b-lactams resistance, the strain is resistant to ciprofloxacin, levofloxacin and erythromycin but is sensitive to gentamicin, rifampicin and tetracyclines. The other two, less prevalent MRSA clones (6.6% and 1.9% of all isolates, respectively), were t008 (SCCmec type IV) and 001 (SCCmec type I). Our hospital is the first in Italy, where t242 reached an endemic level.

Clonal study of methicillin-resistant Staphylococcus aureus isolates of nosocomial origin in a Spanish hospital

E. Baos*, G. Morales, J. Picozo (Madrid, ES)

Objectives: Analysis of susceptibility to a wide panel of antibiotics of MRSA strains obtained from hospitalised, infected patients. Restriction fragment analysis by PFGE of the strains to assign them to clones. Identification of predominant circulating clones.

Methods: 1-Selection of MRSA strains obtained from different types of clinical samples: Identification of strains and antibiotic susceptibility determination were made using WIDER panels and VITEK cards. MIC90 was determined by E-test and dilution in agar following CLSI indications. In the case of vancomycin MICs were read at 24 and 48 hours for detection of putative VISA strains.

2-Clonal analysis: Total DNA of each MRSA strain was digested with Sma1 and analyzed by PFGE. Macerorestriction profiles obtained were analyzed and compared with FPQuest software (Bio-Rad) using the Dice correlation coefficient and the UPGMA with 1% band tolerance. A similarity cut-off of 80% and the criterion of a difference ≤6 bands, were used to define a cluster.

Results: All strains presented MIC90 > 512 to oxacillin, cefoxitin, cefotaxime, ceftazidime, cefepime, erythromycin and clindamycin. MIC90 was 64 to gentamicin and 16 to amikacin and levofloxacin. All strains analyzed were sensitive to glycopeptides, linezolid, tigecycline and rifampicin. Comparative analysis of electrophoretic patterns showed high variability among the strains although they could be clustered into three main groups according to their band similarity. The predominant cluster contained eight strains that were obtained in the ICU. Correlation between clonal relationship and hospital ward origin in other cases was also found.

Conclusion: MRSA strains analyzed presented high level of antibiotic resistance as well as multiresistance as expected because of their nosocomial origin. There is association among clonal groups and hospital ward origin what points to several and distinct reservoirs of MRSA in our hospital. Determination of the circulating clones will facilitate the control and prevention of nosocomial outbreaks.

The occurrence of Panton-Valentine leukocidin in MRSA strains isolated from hospitalised patients in Slovakia

V. Kmet, D. Ohlasova, M. Nitsk * (Kosice, Bratislava, SK)

Objectives: The occurrence of MRSA in Slovakia is increasing. In 2003 community and hospital MRSA represented 3% and 6%, respectively. In 2008 already 8% of community and 13% of hospital S. aureus isolates...
were MRSA. In ICUs reached MRSA proportion in 2008 22% (Slovak National AMR Database www.nars.sk). To characterise hospital MRSA and to detect a possible participation of community strains on hospital invasive infections we have evaluated the Panton-Valentine leukocidin (PVL) genes and identified staphylococcal chromosomal cassette types in current MRSA hospital isolates.

**Methods:** During year 2008 75 methicillin resistant S. aureus isolated from soft tissue abscesses and chirurgical wounds in inpatients (1 per patient) from 7 county hospitals around the country were obtained and analyzed. PVL gene ( lukF-PV, lukS-PV), mecA gene and SCCmec types were determined by PCR. For phenotypic methicillin resistance detection and antibiotic resistance determination, CLSI (2008) criteria were used.

**Results:** All of 75 strains phenotypically determined as MRSA possessed the mecA gene. PVL positive strains represented 14.6% (11/75). Single PVL positive MRSA strain possessed SCCmec type IV cassette and was resistant only to erythromycin. In this case the MRSA was isolated from a patient who was treated with erythromycin. SCCmec type II was not typeable. Of 64 PVL negative MRSA strains, in 22 SCCmec cassette could be determined. 18 of them possessed type II SCCmec cassette and were phenotypically resistant also to erythromycin, clindamycin and ciprofloxacin. Resistance to chloramphenicol could be detected in only three strains. All of 75 tested MRSA strains were phenotypically susceptible to linezolid.

**Conclusions:**
1. The occurrence of PVL genes in current invasive MRSA strains isolated from hospitalised patients in Slovakia is around 14.6%.
2. In this study only one PVL+ MRSA strain possessed SCCmec type IV cassette and most probably originated from community.
3. The predominating SCCmec type in PVL negative and SCCmec typeable invasive MRSA was II. All these strains were resistant also to erythromycin, clindamycin and ciprofloxacin.
4. Of all 75 analysed MRSA strains only three were resistant to chloramphenicol. The linezolid resistance in MRSA strains from Slovak hospitals was not recorded.

**Table 1. Species identification of CNS, clones and toxin genes carriage**

<table>
<thead>
<tr>
<th>Specie (no. of strain)</th>
<th>Clones (no. of strain)</th>
<th>Positive toxin genes (no. of strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis (186)</td>
<td>a (63)</td>
<td>11 4 6 1 1 2</td>
</tr>
<tr>
<td></td>
<td>b (14)</td>
<td>2 2 2 2 2 2</td>
</tr>
<tr>
<td></td>
<td>c (10)</td>
<td>1 1 3 3 3 3</td>
</tr>
<tr>
<td></td>
<td>Others (15 clones 19 strains)</td>
<td>2 1 3 3 3 3 1</td>
</tr>
<tr>
<td>S. haemolyticus (59)</td>
<td>b (44)</td>
<td>10 5 1 1 1 1</td>
</tr>
<tr>
<td></td>
<td>Others (9 clones 15 strains)</td>
<td>1 3 3 1 1 1 3</td>
</tr>
<tr>
<td>S. hominis (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others (6 clones 8 strains)</td>
<td>1 1 1 1 1 1 1</td>
</tr>
<tr>
<td>S. saprophyticus (1)</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>S. simulans (3)</td>
<td>3 clones</td>
<td></td>
</tr>
<tr>
<td>S. lugdunensis (2)</td>
<td>2 clones</td>
<td></td>
</tr>
<tr>
<td>Total: 180</td>
<td>45 clones</td>
<td>25 16 0 15 3 2 4</td>
</tr>
</tbody>
</table>

**P1578 Clones and toxin genes’ carriage of coagulase-negative staphylococci isolated from bacteraemic infants hospitalised in an intensive care unit**


**Objective:** Coagulase-negative staphylococci (CNS), especially *Staphylococcus epidermidis* and *S. haemolyticus*, are the leading causative agents of neonatal nosocomial sepsis. High prevalence of resistance to methicillin and other antistaphylococcal agents is observed among CNS (MR-CNS), a key factor for persistence in neonatal intensive care units (NICUs). An extracellular polysaccharide intercellular adhesin (PIA) encoded by the ica operon has received much concern in biofilm formation. Of clinical importance is the production of TSST-1 and entersotoxins acting as superantigens by CNS. The aim of the present study was to investigate MR-CNS clonal dissemination in the NICU of our University Hospital during a two-year period in correlation to toxin genes’ carriage and the presence of ica operon with biofilm formation.

**Methods:** A total of 180 CNS from 69 patients with bacteraemia were identified at species level by the API Staph System (bioM´erieux) and by restriction fragment length polymorphism of the amplified tuf gene. The MICs of oxacillin, linezolid, vancomycin and teicoplanin were determined by the Etest (Ab Biodisk). Susceptibility against antistaphylococcal agents was tested by the disk diffusion method. The presence of mecA gene, icaA and icaD (ica operon), tst (TSST-1) and the enterotoxins’ genes sea, seb, sec and sed was tested by PCRs. The presence of PVL, sem/seg (of the enterotoxin gene cluster egc) and agr groups (lukS and lukF (encoding PVL), tst (encoding toxic shock syndrome toxin), sem/seg (of the enterotoxin gene cluster egc) and agr groups were determined by the PFGE of chromosomal DNA Smal digests and MLST. CA-MRSA were isolated from patients without any predisposing risk factors.

**Results:**
Four hundred and seventy-three MRSA were recovered from infants, and 327 from children. The majority were CA-MRSA (80% among adults and 99% from children). Genes encoding PVL were detected in 408 strains (80%) from adults and 312 (95%) from children, belonging to ST80 and ST377 clones. Genes of the superantigens’ family (tst and/or egc) were identified among 11% and 7% of adults and children, respectively. PVL-negative strains were classified into ST239, ST30, ST22, ST225, ST585 clones. Multi-resistant strains were identified among adults.

**Conclusions:** PVL-positive MRSA are widely distributed in Greece, mainly among children and to less extend in the adults, causing soft skin and tissue infections but also osteomyelitis and pneumonia. Most of the cases are community-associated belonging to two clones, reinforcing
Belgian MRSA population: nursing homes versus hospital isolates (2007)

C. Wildemauw*, D. De Brouwer, P. Bifani, C. Godard, M. Dehem, R. Joseph, D. Van Kerkhoven, R. Vanhoof (Brussels, BE; Lille, FR; Ypres, Sint-Truiden, BE)

Objectives: Staphylococcus aureus is a long-standing subject of epidemiological investigation typically employing a variety of (molecular) techniques: methicillin-resistant S. aureus (MRSA) receiving the most attention. In Belgium, in 2006, the MRSA proportion at hospital level was estimated 24% (EARS-S). Considering the global and dynamic nature of MRSA, continued surveillance is important to more clearly understand the role of potential reservoirs, such as in the stream of elderly patients coming from nursing homes, in hospital MRSA population. Therefore, we looked for a possible correlation between hospital and nursing home MRSA.

Methods: We compared 179 MRSA isolates, collected in Belgium by a medical laboratory (requests from 22 nursing homes; N=74) and by a general hospital (N=105) between June and December 2007. We used phage- and spa-typing as well as PCR detection of the Panton-Valentine leukocidin (PVL) gene.

Results: In 2007, in the nursing homes, 70% of the MRSA population belonged to phage type group O* and 9% to J*. The remaining isolates (19%) could not be assigned to a defined group. Nineteen different spa types (Ridom StaphType) were found: 55% belonged to O* and 4% to J*. Fifteen different spa types were PVL positive, all of spa type O38. In the hospital, 35% of the isolates belonged to the phage type group J*, 23% to O* and 39% could not be put in a defined group. Eighteen different spa types were found: 30% belonged to O38, 29% to J121, 13% to O38 and 10% to O02. Three spa types were only represented in two isolates and eleven spa types were unique. Four (5.41%) isolates were PVL positive, all of spa type O38. In the hospital, 35% of the isolates belonged to the phage type group J*, 23% to O* and 39% could not be put in a defined group. Eighteen different spa types were found: 30% belonged to O38, 29% to J121, 13% to O38 and 10% to O02. Three spa types were only represented in two isolates and eleven spa types were unique. Only one (0.95%) isolate was PVL positive.

In both MRSA populations, the dominant phage type groups were O* and J*. spa types O38, J740 were frequent and only few PVL positive isolates were found. Significant differences between the MRSA populations from the nursing homes and the hospital were found in the percentages of phage type groups and dominant spa types.

Conclusion: The “super bug” MRSA has caused increasing problems in nursing homes and hospitals. Some concordances were found between 1997 and 2008 by PFGE prepared in agarose blocks and digested with Smal. Other molecular typing methods were performed (MLST; SCCmec typing method previously described by Oliveira et al.) to a representative subset of MRSA isolates.

Results: From 1997 to 2000 only two clones were present in our hospital, the Iberian clone (PFGEtype A, ST247-MRSA-I) with a clear predominance (between 67 and 93% of the MRSA isolated in this period), and the Paediatric clone (PFGEtype B, ST5-MRSA-IVA). In 2000, the Iberian clone was the predominant one (66%) but other two MRSA clones were isolated the New York/Japan clone (PFGEtype D, ST5-MRSA-II variant) and the EMRSA-16 clone (PFGEtype E, ST32-MRSA-II). From 2001 to 2005, EMRSA-16 clone was the predominant one (between 68 and 74% of the MRSA isolated in this period) replacing the Iberian clone. In 2003, EMRSA-15 (PFGEtype F, ST22-MRSA-IV) appears in our hospital for the first time. In the last three years (2006–2008) the EMRSA-15 and the Paediatric clone have increased little by little replacing the EMRSA-16 clone. In 2008 these two clones were the 82% of the MRSA isolated. A SCCmec type IIA was observed in one MRSA isolated in 2008 with a new PFGE profile. We are trying to confirm by MLST that we have the first Brazilian clone isolate in our hospital.

Conclusions: The picture of the clones circulating in our hospital has completely changed, starting with a predominance of the Iberian clone practically eliminated in the last years, following with a period of EMRSA-16 clone dominance and currently with a co-dominance of EMRSA-15 and Paediatric clone. More microbiological and epidemiological studies are necessary to explain these clonal changes.

Community-onset methicillin-resistant Staphylococcus aureus in Belgium: changing epidemiology and clone diversification


Objectives: Panton-Valentine Leukocidin (+) community-onset methicillin-resistant S. aureus (CO-MRSA) infections are reported in Belgium since 2003. The objectives of this study were to update the demographic characteristics and molecular epidemiology of cases of CO-MRSA colonisation and infection in Belgium over the last 3 years.

Material and Methods: CO-MRSA was defined as MRSA detected in outpatients in or in the first 48h after hospital admission. From 2005 to 2007, 120 CO-MRSA strains were referred for characterisation together with epidemiologic data. MRSA were confirmed by PCR for 16S rRNA, nuc and mecA genes. Strains were genotyped by Smal macrorestriction, SCCmec typing and MLST. Multiplex PCR was used to detect genes encoding PVL, TSST-1, exfoliatin A, B and D, enterotoxin A to R and resistance to tetracyclines, aminoglycosides and macrolides-lincosamides-streptogramins.

Results: Among CO-MRSA strains, 77 (64%) isolates were PVL(+). Patients with MRSA PVL(−) had a bimodal age distribution with very young and old patients whereas patients with PVL(+) strains had a unimodal age distribution. Compared with PVL(−) strains, PVL(+) strains more frequently associated (p < 0.05) with skin and soft tissue infections (79% vs 39%) and with recent travel abroad (26% vs 9%). Compared with PVL(+) strains, PVL(−) strains were often associated (p < 0.05) with asymptomatic carriage (28% vs 7%) and previous hospitalisation (37% vs 17%). Cross infection was limited to a small cluster of PVL(−) cases (n=10) in a maternity ward. PVL(+) strains belonged predominantly to genotypes: PFGE X-ST00-SCCmec IV (n=47), J-ST30-SCCmec IV (n=6) and A-ST6-SCCmec IV (n=7). Five A-ST6-SCCmec IV strains carried the araC gene of the ACME gene cluster (USA300-marker) One of these was from a patient who had travelled to the USA. PVL(+) strains showed a higher PFGE type diversity and more frequently harboured enterotoxin genes than PVL(+) strains. The distribution of enterotoxin and resistance genes was strongly linked to PFGE type.

Conclusion: CO-MRSA strains from Belgium showed clonal diversification in 2005–2007. PVL(+) were strains associated with recent travel and skin and soft tissue infections whereas PVL(−) strains were associated with asymptomatic carriage and healthcare exposure. PVL(+) MRSA strains predominantly belonged to the European ST30 clone. The spread of the USA300 clone is of concern.

Evolution of the MRSA clones at a university hospital in the Canary Islands

I. Montesinos, B. Castro*, Y. Barroso, T. Delgado, M.J. Ramos, M. Lecuona, A. Sierra (La Laguna, ES)

Objectives: The aim of this study is to present the evolution of the MRSA clones circulating in the University Hospital of Canary Islands (HUC) between 1997 and 2008.

Methods: The HUC is a tertiary-care hospital of 650 beds with a high MRSA endemicity. We have studied a total of 1192 MRSA isolates between 1997 and 2008 by PFGE prepared in agarose blocks and digested with Smal. Other molecular typing methods were performed (MLST; SCCmec typing method previously described by Oliveira et al.) to a representative subset of MRSA isolates.

Results: From 1997 to 2000 only two clones were present in our hospital, the Iberian clone (PFGEtype A, ST247-MRSA-I) with a clear predominance (between 67 and 93% of the MRSA isolated in this period), and the Paediatric clone (PFGEtype B, ST5-MRSA-IVA). In 2000, the Iberian clone was the predominant one (66%) but other two MRSA clones were isolated the New York/Japan clone (PFGEtype D, ST5-MRSA-II variant) and the EMRSA-16 clone (PFGEtype E, ST32-MRSA-II). From 2001 to 2005, EMRSA-16 clone was the predominant one (between 68 and 74% of the MRSA isolated in this period) replacing the Iberian clone. In 2003, EMRSA-15 (PFGEtype F, ST22-MRSA-IV) appears in our hospital for the first time. In the last three years (2006–2008) the EMRSA-15 and the Paediatric clone have increased little by little replacing the EMRSA-16 clone. In 2008 these two clones were the 82% of the MRSA isolated. A SCCmec type IIA was observed in one MRSA isolated in 2008 with a new PFGE profile. We are trying to confirm by MLST that we have the first Brazilian clone isolate in our hospital.

Conclusions: The picture of the clones circulating in our hospital has completely changed, starting with a predominance of the Iberian clone practically eliminated in the last years, following with a period of EMRSA-16 clone dominance and currently with a co-dominance of EMRSA-15 and Paediatric clone. More microbiological and epidemiological studies are necessary to explain these clonal changes.
**P1582** Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates from nursing homes (NH) of western Switzerland suggests transmission within NH

**L. Senn*, C. Petignat, C. Bellini, E. Masserery, L. Christin, A. Cometta, G. Zanetti, D.S. Blanc (Lausanne, Nyon, Yverdon, CH)**

**Background:** In Western Switzerland, an area of low MRSA prevalence, more than two-thirds of the strains isolated in acute-care hospitals between 1997 and 2007 belong to 4 predominant genotypes. Few data exist on the molecular epidemiology of MRSA in NH and its correlation to the epidemiology of local hospitals. These data should help to determine whether MRSA carriage in NH residents is mainly imported from hospitals or originates from transmission within NH.

**Objectives:** To describe the molecular epidemiology of MRSA carriage among residents of NH in Western Switzerland and to compare this epidemiology with that of the local tertiary care hospital (TCH).

**Methods:** **Study population:** i) in NH: all MRSA-positive residents identified through a point prevalence survey conducted in 130 NH of Western Switzerland (Oct.-Mar. 08); ii) in TCH: all newly identified MRSA-positive patients in 2007.

**Molecular typing:** double locus sequence typing (DLST) − a new typing strategy for MRSA that uses the repeat sequences of clfB and spa genes − was performed on 1 isolate per patient.

**Results:** In NH, we identified 44 genotypes in 273 residents, of which i) 2 were predominant: DLST 2–2 and 3–3 found in 163 (60%) and 42 (15%) residents, respectively; ii) 11 (37 residents − 14%) belonged to small clusters of 2 to 7 residents; and iii) 31 (11%) were unique. In TCH, we identified 42 genotypes in 235 patients, of which i) 3 were predominant: DLST 2–2, 3–3 and 4–4 found in 128 (54%), 24 (10%), and 19 (8%) patients, respectively; ii) 17 (42 patients − 18%) belonged to small clusters of 2 to 3 patients; and iii) 22 (9%) were unique.

Only 7 genotypes were recovered from NH residents and from TCH patients, of which 2 were predominant (DLST 2–2 and 3–3). The third predominant TCH genotype (DLST 4–4) was significantly less frequent in NH than in TCH, and all other genotypes were specific to either NH or TCH. These results suggest that MRSA carriage in NH residents is not only imported from TCH. Moreover, among 11 genotypes involved in small clusters of NH residents, 10 were not recovered in TCH patients, suggesting transmission within NH.

**Conclusion:** Molecular epidemiology of MRSA in NH of Western Switzerland suggests that MRSA carriage in NH residents is mainly imported from hospitals, but also originates from transmission within NH.

**P1583** Temperature affects ccrAB-mediated excision of SCCmec IV in USA300 and USA400 community-associated *Staphylococcus aureus* strains

**J. Black, R.V. Goering* (Omaha, US)**

**Objectives:** A variety of studies have suggested that the relatively small size of SCCmec IV may facilitate its horizontal transfer contributing to the emergence of community-associated MRSA. Encoded within SCCmec, ccrAB recombinase activity is envisioned as important in this process mediating site-specific cassette integration and excision. However, with regard to *S. aureus* strain USA400 (MW2) the literature is conflicted with reports of both normal and defective SCCmec excision (Janssen et al., 2006; Antimicrob. Agents Chemother. 50:2072–78; Noto and Archer, 2006; Antimicrob. Agents Chemother. 50:2782–88, respectively) the latter attributed to an interfering downstream enterotoxin H sequence. To clarify this issue we examined both CA-MRSA 400 (MW2) and USA300 strains for site-specific excision of SCCmec and, in the case of USA300, the arginine catalytic mobile element (ACME).

**Methods:** Site-specific excision of SCCmec and ACME were assessed by both conventional and real-time PCR employing primers designed to detect and amplify chromosomal, SCCmec, and ACME junctions generated due to excision. Experiments were conducted with DNA templates prepared from cells grown at 25°C, 37°C, and 45°C to examine the potential influence of temperature on the excision process.

**Results:** In both USA300 and USA400 *S. aureus* strains, site-specific SCCmec excision was readily detected at 25°C but was markedly reduced at increased temperatures. Similarly, in USA300, excision of the ACME “cassette” either alone or in combination with SCCmec also occurred in a temperature-dependent manner. ACME excision was not observed in USA300 strains which had lost SCCmec confirming the importance of ccrAB recombinase activity in the process.

**Conclusions:** Site-specific excision of SCCmec IV appears to readily occur in both USA300 and USA400 *S. aureus* strains but with reduced efficiency at temperatures above 25°C. The ability of ACME to excise (alone or in combination with SCCmec) only in the presence of ccrAB recombinase favours a model of CA-MRSA (e.g., USA300) evolution where SCCmec is acquired first, followed by ACME, rather than vice versa.

**P1584** Geographic dissemination of methicillin-resistant *Staphylococcus aureus* spa-types in hospitals and rehabilitation clinics in the Dutch-German EUREGIO MRSA-net

**R. Kick*, A. Mellmann, K. Becker, B. Mulderdorpp, I. Daniels-Haardt, A. Jarke, R. Hendrix, A. Friedrich (Munster, DE; Enschede, NL)**

**Objectives:** Hospitals and laboratories within the Dutch-German quality network EUREGIO MRSA-net have agreed to use protein A (spa) typing as a common area-wide standard for the molecular characterisation of MRSA. A geographic information system (GIS) was developed for the visualisation of differences in the regional spread of MRSA clones. Here we describe changes in the regional distribution of MRSA spa types obtained between 2006 and 2008.

**Methods:** Each first MRSA isolated either from screening swabs or from clinical specimens obtained from patients of 36 regional German and 4 regional Dutch hospitals (representing 94% and 100% of all regional beds, respectively) between 2006 and 2008 was spa sequence-typed and entered in the GIS database. Differences in the distribution of spa types between different regional districts and facilities were calculated using chi-square, Fisher exact or t-test where appropriate.

**Results:** The GIS database (data retrieval 06.01.2009) includes 1,780 German MRSA isolates from 2006 (including 31 blood cultures, BC), 2,539 from 2007 (including 50 BC) and 2,654 from 2008 (including 27 BC). Furthermore, the database contains 18, 57 and 71 Dutch MRSA isolates (n=0 BC) isolated 2006, 2007 and 2008, respectively. The German isolates were assigned to a total of 105 different spa types in 2006, 131 in 2007 and 169 in 2008. Among the Dutch isolates 9 spa types were found in 2006, 18 in 2007 and 15 in 2008. While the predominating spa types among the German isolates (% of all isolates) were represented by t003 (34%), t032 (27%), t011 (6%), t004 (4%) and t034 (3%), the predominating Dutch spa types were t011 (40%), t1458 (10%), t1108 (9%), t026 (7%) and t008 (7%). Overall, the distribution of the major spa types varied significantly on a local level, between different German districts and across the border. The GIS indicated geographic and temporal clusters.

**Conclusion:** The EUREGIO MRSA-net GIS allows an area-wide and cross-border molecular surveillance of MRSA. The genotypic background of MRSA isolates distributed in 40 different hospitals in the Dutch and the German part of the EURREGIO varies significantly with respect to spa types. The implementation of the newly established MRSA network-associated GIS facilitates the detection of outbreaks and clusters for local infection control staff and rapidly indicates emerging clones with epidemic potential.
**P1585** Emergence of a methicillin-resistant *Staphylococcus aureus* in Spain

S. Molinos, R. Ehricht, M. Giménez*, S. Monecke, C. Rodrigo, C. Prat, N. Sopena, V. Ausina (Badalona, ES; Jena, Dresden, DE)

Objectives: Community-associated MRSA (CA-MRSA) disease usually presents as pyogenic skin and soft tissue infections (SSTIs) in previously healthy individuals. USA300, ST-8 is the most prevalent clone in this country and contains a genomic island, known as arginine catabolic mobile element (ACME), which encodes an arginine deaminase. This enzyme may enhance the virulence of USA 300 by enabling it to colonise and evade host defences.

In Spain, CA-MRSA isolates don’t belong to the main European clone ST30, but to the ST8. Here, we report the first three Spanish isolates of the CA-MRSA USA300 ST-8 clone without the ACME-argA gene in their genetic background.

Methods: DNA microarrays based on the Array-Tube platform (CLONDIAG, Jena, Germany) were used for genotyping three isolates from two children with superficial skin infections and one adult with septic arthritis and bacteremia (Table 1).

Results: Hybridisation patterns for the ag-gene-specific probes showed that three isolates matched to agr type I, carried a type IV staphylococcal cassette (SCC) and belonged to CA-MRSA of clonal group ST8. All isolates contained the genes for both toxin Panton-Valentine leukocidin (PVL) components (lukF-PV and lukS-PV). Moreover, the three isolates also lack ACME-argA gene. In addition, all isolates contained the cch gene for chemotaxis inhibitory protein (CHIPS).

Conclusion: The three isolates of our patients lacked the ACME. To date, these are the first Spanish isolates of ACME-negative USA300. Our study shows the emergence and spread of a clone that could potentially change the clinical spectrum of *S. aureus* infections in our community; thus, the comparison of the virulence content of well characterised isolates causing different clinical syndromes is crucial in predicting the clinical outcome and the most accurate treatment for CA-MRSA infections.

**P1586** ST22 methicillin-resistant *Staphylococcus aureus* is a major epidemic clone circulating in hSR

R. Baldan*, M. Moro, P. Ciccheri, D. Cirillo (Milan, IT)

Objectives: In the late 1990s, highly virulent Meticillin-Resistant *Staphylococcus aureus* (MRSA) clones, the major cause of nosocomial infections, emerged in the community, causing infections in healthy people, without predisposing risk factors. Community-acquired MRSA (CA) differ from Hospital-acquired (HA) for the genetic background on the staphylococcal cassette chromosome mec (SCCmec), susceptibility to non-β-lactams and virulence. In order to investigate the presence of CA-MRSA and to characterise MRSA major clones in a large nosocomial setting, we investigated at the molecular level all clinically significant MRSA strains isolated at the hSR from 2006.

Methods: We tested 275 MRSA for SCCmec type, PVL and other toxins genes and we performed cluster analysis by Pulsed-Field Gel Electrophoresis after Small restriction, spa and multilocus sequence typing.

Results: 48% of isolates collected carried SCCmec typical of hospital strains, predominantly SCCmec I (44.7%), while 41.5% carried SCCmec IV (V in one case) typical of CA-MRSA and was more susceptible to non-β-lactams than HA-MRSA; 10% was not or partially typeable but had susceptibility pattern typical of CA-MRSA. Eight strains were PVL-positive and 6 of them (5 SCCmec IV and 1 V), considering clinical data, were identifiable as CA-MRSA: spa, MLST and PFGE of 5 of them showed their relatedness to the epidemic MRSA clone USA300. Cluster analysis of SCCmec I MRSA showed 4 major clades with several established pulsetypes. Cluster analysis of SCCmec IV MRSA showed 2 major clades: 1 of them represented the 29% of all isolates or >65% of SCCmec IV MRSA. PFGE identified this clone as ST22 EMRSA15, a major epidemic HA-MRSA clone circulating in the UK hospitals. SCCmecIV MRSA isolated from blood cultures increased from 35% in 2006 to 50% in 2008.

Conclusion: 41.5% of MRSA circulating at the hSR carried SCCmec IV and >65% of them had characteristics of the epidemic UK EMRSA15 ST22; we report that 2.2% of the strains are CA-MRSA PVL positive, according to European data. Considering isolates obtained from sepsis in the last 3 years, we observed an increase of MRSa SCCmec IV from 35% to 50%.

**P1587** Molecular characterisation of methicillin-resistant *Staphylococcus aureus* in Cape Town hospitals

V. Madikane*, M.J. van Rensburg, M. Chachage, A. Whitelaw, G. Elisha (Cape Town, ZA)

Objective: The objective of the study was to gain an understanding of the molecular epidemiology and SCCmec content of clinical MRSA strains in hospitals in Cape Town.

Methods: A total of 36 MRSA strains, isolated from patients in four hospitals in Cape Town, were characterised. Pulsed field gel electrophoresis (PFGE) was used to study the relatedness of the MRSA and multiplex PCR assays were carried out to determine their staphylococcal chromosomal cassette mec (SCCmec) content. Antibiotic susceptibility profiles of the MRSA were analysed with respect to genetic background and SCCmec type.

Results: Using a threshold of 90% similarity, 7 PFGE clusters were identified: 30 MRSA were assigned to either cluster 3 or 5. When clusters 1, 3, 5 and 7, containing 4 or more strains, were stratified by hospital, clusters 1 and 3 were identified in Groote Schuur (GSH), Red Cross War Memorial Children’s (RCCH) and Victoria (VH) hospitals. Cluster 5 was observed in MRSA from Mowbray Maternity hospital (MMH) as well as GSH and RCCH. MRSA strains belonging to cluster 7 were isolated only from GSH, and RCCH. SCCmec types I and IV were identified in 24 and 26 strains, respectively. SCCmec types II and III were observed in 4 and 2 strains, respectively. Grouping the mec types in PFGE clusters showed that cluster 5 comprised only SCCmec type I strains (17/24). Similarly, SCCmec type IV strains were unique to cluster 3 (13/26) and 1 (6/26). The SCCmec type II and III strains belonged to cluster 7 and 6, respectively. There were striking differences between the antimicrobial susceptibility profiles of SCCmec IV containing MRSA and the SCCmec I strains. Resistance to gentamicin, co-trimoxazole, ciprofloxacin and rifampicin was nearly universal in the 26 MRSA type IV strains. Only 10 of these isolates were resistant to erythromycin and clindamycin. In contrast, almost all of the MRSA SCCmec type I strains were resistant to erythromycin and clindamycin (21/24) but susceptible to the other antibiotics.

Conclusions: Related MRSA strains were identified across hospitals in Cape Town, suggesting a common epidemiology and transmission of MRSA either by patients or by healthcare workers. Over half of the MRSA segregated in two PFGE clusters, which also accommodated the majority of the two most frequently detected SCCmec types, I and IV. A majority of the SCCmec type IV strains were resistant to a spectrum of antibiotics.
**The use of Raman spectroscopy in epidemiology of methicillin-resistant Staphylococcus aureus**

M.W.H. Wulf*, H.F.M. Willemse-Enix, C.M. Verdun, A. van Belkum, K. Maquelin (Veldhoven, Rotterdam, NL)

**Objective**: The Netherlands has an active search and destroy policy for methicillin resistant Staphylococcus aureus (MRSA). In order to maintain this policy, a reliable and quick typing method for isolates is essential. The objective of this study was to see if Raman spectroscopy provides such a method for normal- and livestock-associated MRSA.

**Methods**: Between 2002 and 2007 a total of 433 MRSA positive subjects were identified. Of these a total of 392 isolates were analysed using Raman spectroscopy. Spectroscopic fingerprints were obtained using a dedicated Raman spectrometer, requiring approx. 40 seconds per sample. Cluster analysis on these fingerprints was performed using the pair wise correlations as a distance measure in combination with Ward’s cluster algorithm.

Results were compared with PFGE typing results obtained from the national reference library and with epidemiological data.

**Results**: Of the 403 isolates analysed, 157 were non-typable by PFGE. The remaining 246 represented a total of 51 different PFGE types. Raman typing resulted in a total of 86 Raman clusters. Of the subjects, 83 were unexpected MRSA cases, 150 came from targeted screening of known risk groups, 11 were family members of MRSA positive patients and 135 came from outbreak screening cultures. Further analyses of 20 clusters of epidemiologically linked isolates (n=154) showed that 128 (82%) had identical PFGE clusters. Raman typing of the same isolates showed that 126 (81%) had corresponding Raman clusters. Of the 28 mismatches, 13 (4.4%) isolates had both a different Raman and PFGE of the outbreak strain.

Analyses of PFGE non-typable isolates showed that in one outbreak with 10 cases, all had identical Raman types, in one case of possible transmission, 2 Raman clusters were seen. An overall analyses of the PFGE non-typable isolates in our collection (n=157) resulted in 24 different Raman clusters.

**Conclusions**: Raman spectroscopy is a reproducible method for typing of MRSA for epidemiological typing. The results obtained with this technique are comparable to PFGE and in good concordance with the epidemiological data.

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**Antimicrobial susceptibility of tigecycline**

A. Burillo*, Y. Gil, F. López-Fubal, J.L. Gómez-Garcés (Madrid, ES)

**Objectives**: To explore the possibility of restoring the activity of currently available antimicrobials using different combinations of these agents.

**Methods**: Time-kill curves of tigecycline plus imipenem were determined for one strain of a multi-drug resistant P. aeruginosa in which resistance mechanisms were characterised genetically: the strain overexpressed AmpC and the efflux pump MexEF-OprN, had point mutations in oprD and was metallo-β-lactamase-nonproducing (MBL-). The MIC of each antibiotic for this strain was 64 mg/L. Tests were conducted using: a) the corresponding MICs of both antimicrobials; b) a 2 mg/L concentration of each drug; and c) a tigecycline concentration of 2 mg/L (equivalent to the physiological concentration reached in tissues) along with the MIC of imipenem.

**Results**: At antibiotic concentrations equal to 1×MIC of tigecycline and imipenem, a synergistic and action-restoring effect was produced from 24 hours onwards. At low antibiotic concentrations relative to the MIC of 0.03×MIC, the synergistic and action-restoring effects seen at higher antibiotic concentrations persisted, although a lower decrease in the number of microorganisms of 1−1.5 log10 was observed. Finally, concentrations of tigecycline as low as 0.03×MIC (2 mg/L) when combined with imipenem were able to rescue the activity of imipenem against this highly imipenem-resistant P. aeruginosa strain (Figure 1).

The effect observed was bactericidal with an inoculum reduction of >3 log10 units.

**Conclusions**: Our results point to the potential use of tigecycline in combination with carbapenems to combat multiresistance in MBL-P. aeruginosa.

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**Activity of tigecycline against Acinetobacter spp. from various specimen sources collected in Europe over the past five years**


**Objective**: In 2006, tigecycline (TIG) was approved in Europe (EU) for treatment of complicated skin and skin structure infections and complicated intra-abdominal infections, infections which can be caused by Acinetobacter spp (AC). AC infections can be difficult to treat due to the multi-drug resistance commonly encountered with this pathogen. Additionally, the activity of antimicrobials used to treat these infections can vary based on the site of infection. The current study examined the in vitro activity of TIG against AC isolated in Europe for the purpose of detecting any differences in activity pre- and post-launch of this recently approved agent and any variation in the activity profile against isolates from prevalent specimen sources for AC infection (respiratory, blood, urine, and skin/wound).

**Methods**: In total, 272 AC isolates were obtained from 31 hospitals in 11 countries across EU from 2003 to 2008. Isolates were tested centrally by broth microdilution (CLSI M7-A7). There are currently no EUCAST TIG interpretive breakpoints (BP) for AC; therefore, EUCAST Enterobacteriaceae BPs was used to interpret TIG MIC results.

**Results**: See the table.

<table>
<thead>
<tr>
<th>Year of isolation</th>
<th>Specimen source</th>
<th>TIG MIC (mg/L)</th>
<th>%S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mode</td>
<td>MIC50</td>
</tr>
<tr>
<td>2003</td>
<td>40</td>
<td>0.12–2</td>
<td>1</td>
</tr>
<tr>
<td>2005</td>
<td>47</td>
<td>0.06–2</td>
<td>0.25</td>
</tr>
<tr>
<td>2006</td>
<td>87</td>
<td>0.06–4</td>
<td>1</td>
</tr>
<tr>
<td>2007</td>
<td>78</td>
<td>0.06–4</td>
<td>0.5</td>
</tr>
<tr>
<td>2008</td>
<td>20</td>
<td>0.06–2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>104</td>
<td>0.06–4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>0.06–2</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>0.06–2</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.06–4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Isolates of unknown specimen source were excluded.

**Conclusions**: TIG maintained potent in vitro activity against AC. The TIG MIC50 (0.25–0.5 mg/L) and MIC90 (1 to 2 mg/L) remained consistent, regardless of the year isolated or the specimen source. Due
In vitro activity of tigecycline against pathogens isolated from most common body sites – Eastern European Data – T.E.S.T. Program 2008


Background: Tigecycline (TIG), a new glycycline, has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the in vitro activity of TIG and 10 comparators against respective Gram positive/negative species. For the overall T.E.S.T. program isolates were collected from 205 hospital sites in 30 countries from 2004 to 2008.

Methods: In this survey, clinically significant isolates from Eastern European testing sites (Poland, Hungary, Greece, and Latvia) were analyzed. The isolates were identified to the species level at the participating sites and confirmed by the central laboratory. MICs were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines.

Results: TIG activity against selected pathogens and body sites are shown in the table.

<table>
<thead>
<tr>
<th>Blood</th>
<th>Respiratory</th>
<th>Urinary tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%S MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>n</td>
</tr>
<tr>
<td>E. coli</td>
<td>26</td>
<td>100</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>9</td>
<td>na</td>
</tr>
<tr>
<td>S. aureus</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>17</td>
<td>na</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>1</td>
<td>na</td>
</tr>
</tbody>
</table>

*na = breakpoints not available.

Conclusions: Tigecycline showed excellent inhibitory activity against all groups of pathogens regardless of isolation site. Tigecycline MIC<sub>90</sub> of <1 µg/ml against Gram positive pathogens (including resistant phenotypes) and MIC<sub>90</sub> of ≤2 µg/ml against Enterobacteriaceae and Acinetobacter spp. validate the potent inhibitory activity of TIG against Eastern European community/hospital pathogens.

In vitro activity of tigecycline against inpatient and outpatient pathogens from centres in Pacific Rim countries – a multi-year update

D. Hohan, M. Renteria, J. Johnson, R. Budal*, S. Hawser, M. Hackel, S. Bouchillon, B. Johnson, D. Dowzicky (Schaumburg, Collegeville, US)

Background: Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections.

Methods: A total of 653 clinical isolates from five Pacific Rim testing sites during 2004 to 2008 were identified to the species level and confirmed by the central laboratory. Minimum Inhibitory Concentration (MIC) values were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines.

Results: Selected results are listed in the tables.

Conclusions: Tigecycline’s in vitro activity was comparable to or greater than most commonly prescribed broad spectrum antimicrobials without any demonstrable change in susceptibility between in- and outpatient bacterial study strains. Tigecycline’s inhibitory activity against Enterobacteriaceae was comparable to imipenem; vs. Acinetobacter spp. tigecycline’s MIC<sub>90</sub> was 32-fold lower than imipenem’s. Against Enterobacteriaceae spp., Tigecycline’s activity was similar to linezolid and vancomycin.

<table>
<thead>
<tr>
<th>Enterobacteriaceae</th>
<th>Acinetobacter spp.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-patients (n=219)</td>
<td>Out-patients (n=83)</td>
</tr>
<tr>
<td>%S MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>%S MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>TIG</td>
<td>98.6</td>
</tr>
<tr>
<td>Amikacin</td>
<td>95.2</td>
</tr>
<tr>
<td>Cefepime</td>
<td>88.2</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>73.7</td>
</tr>
<tr>
<td>Imipenem</td>
<td>100</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>79.4</td>
</tr>
<tr>
<td>Minocycline</td>
<td>80.5</td>
</tr>
<tr>
<td>Pip-Tazo</td>
<td>87.1</td>
</tr>
</tbody>
</table>

*na = breakpoints not available.

TIG MIC<sub>50</sub> calculated if n<10.

A comprehensive analysis of European data from Tigecycline Evaluation Surveillance Trials (TEST) Program, 2004–2008


Background: Tigecycline (TIG), a new glycycline, has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the in vitro activity of TIG and 10 comparators against respective Gram positive/negative species. Isolates were collected from 316 hospital sites in 24 countries from 2004 through 2008.

Methods: 34,401 clinically significant isolates were identified to the species level at participating sites and confirmed by the central laboratory. MICs were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines.

Results: TIG in vitro activity on selected pathogens are shown in the table. Data on resistant phenotypes will be presented.

Conclusions: TIG has been described an expanded broad spectrum antimicrobial because of its consistent activity against Enterobacteriaceae including extended spectrum β-lactamase producers, S. aureus including methicillin-resistant strains, S. pneumoniae including penicillin-resistant strains, both carbapenem-sensitive and -resistant Enterococcus spp., and H. influenzae including β-lactamase producers. TIG wide spectrum of activity promises to provide enhanced antimicrobial coverage of serious nosocomial/community pathogens.

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>TIG MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>%S MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>%S MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>%S MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>%S MIC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>98.6</td>
<td>100</td>
<td>1</td>
<td>0.5</td>
<td>na</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>95.2</td>
<td>8</td>
<td>96.5</td>
<td>4</td>
<td>91.9</td>
</tr>
<tr>
<td>Cefepime</td>
<td>88.2</td>
<td>16</td>
<td>95.6</td>
<td>2</td>
<td>73</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>73.7</td>
<td>&gt;32</td>
<td>92</td>
<td>8</td>
<td>70.3</td>
</tr>
<tr>
<td>Imipenem</td>
<td>100</td>
<td>1</td>
<td>100</td>
<td>1</td>
<td>81.1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>79.4</td>
<td>8</td>
<td>92.9</td>
<td>0.5</td>
<td>70.3</td>
</tr>
<tr>
<td>Minocycline</td>
<td>80.5</td>
<td>8</td>
<td>90.3</td>
<td>4</td>
<td>97.3</td>
</tr>
<tr>
<td>Pip-Tazo</td>
<td>87.1</td>
<td>32</td>
<td>92</td>
<td>16</td>
<td>75.7</td>
</tr>
</tbody>
</table>

*na = breakpoints not available.

A No MIC<sub>90</sub> calculated if n<10.

Cefepime | 88.2 | 16 | 95.6 | 2 | 73 | >32 | 85.7 | – |
Ceftazidime | 73.7 | >32 | 92 | 8 | 70.3 | >32 | 100 | – |
Imipenem | 100 | 1 | 100 | 1 | 81.1 | 16 | 100 | – |
Levofloxacin | 79.4 | 8 | 92.9 | 0.5 | 70.3 | >8 | 100 | – |
Minocycline | 80.5 | 8 | 90.3 | 4 | 97.3 | 4 | 100 | – |
Pip-Tazo | 87.1 | 32 | 92 | 16 | 75.7 | >128 | 100 | – |
**P1594** Susceptibility of Gram-negative/positive pathogens isolated in the United Kingdom and Ireland: a multi-year update

**Background:** The rapid emergence of multi-drug resistant pathogens has undermined the efficacy of many widely used broad spectrum antibacterials and prompted the development of newer antimicrobials. Tigecycline is a new glycyclcline shown to have broad spectrum activity against many hospital pathogens. The purpose of this study was to examine the activity of tigecycline and comparators to nosocomial pathogens isolated in the UK and Ireland between 2004-2008.

**Methods:** A total of 1,321 nosocomial pathogens were identified at each site and confirmed by a reference laboratory. MICs were determined at each site utilising supplied broth microdilution panels and interpreted according to EUCAST guidelines.

**Results:** See the tables.

**Table 1:** Susceptibility of Gram-negative/positive pathogens isolated in the United Kingdom and Ireland: a multi-year update

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>Tigecycline %S MIC&lt;sub&gt;90&lt;/sub&gt; % inhibited at MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Acinetobacter spp. (68)</td>
<td>NA</td>
</tr>
<tr>
<td>Enterobacteriaceae (420)</td>
<td>94</td>
</tr>
<tr>
<td>ESBL producers* (45)</td>
<td>100</td>
</tr>
<tr>
<td>Enterobacter spp. (122)</td>
<td>90</td>
</tr>
<tr>
<td>E. faecalis (41)</td>
<td>95</td>
</tr>
<tr>
<td>E. faecium (29)</td>
<td>100</td>
</tr>
<tr>
<td>H. influenzae (72)</td>
<td>NA</td>
</tr>
<tr>
<td>P. aeruginosa (100)</td>
<td>NA &gt;16 –</td>
</tr>
<tr>
<td>S. aureus (MSSA) (69)</td>
<td>100</td>
</tr>
<tr>
<td>S. aureus (MRSA) (54)</td>
<td>96</td>
</tr>
<tr>
<td>S. pneumoniae (70)</td>
<td>NA</td>
</tr>
<tr>
<td>S. agalactiae (41)</td>
<td>100</td>
</tr>
</tbody>
</table>

*ESBL producing E. coli, K. oxytoca, K. pneumoniae.

**Tigecycline at MICs of 2 or less. Tigecycline promises expanded broad spectrum coverage against multiply resistant pathogens isolated in Italy.**

**P1596** A multi-year update of in vitro activity of tigecycline and commonly used antimicrobials against significant clinical isolates collected from 2004 to 2008 in Belgium

**Background:** Development of bacterial resistance continues to cause concern world-wide, but availability of newer agents offers clinicians options for therapy. Tigecycline (TIG) has a very broad spectrum of activity, including strains resistant to other drugs. As part of the global Tigecycline Evaluation Surveillance Trial, strains collected in Belgium from 2004 to 2008 were evaluated for susceptibility to several antimicrobials.

**Methods:** Strains were collected and identified at 4 sites in Belgium. MICs were determined at each site using custom broth microdilution panels following CLSI guidelines.

**Results:** Tigecycline was the third most active agent against Enterobacteriaceae spp. after imipenem and amikacin, displayed the vancomycin and linezolid, and superior to levofloxacin and minocycline.

**Table 2:** Tigecycline and comparators against nosocomial pathogens in Italy from 2004–2008

**All Gram pos**

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>Tigecycline %S MIC&lt;sub&gt;90&lt;/sub&gt; %S MIC&lt;sub&gt;90&lt;/sub&gt; %S MIC&lt;sub&gt;90&lt;/sub&gt; %S MIC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>E. coli/Kleb</td>
<td>0.5</td>
</tr>
<tr>
<td>Enterococci</td>
<td>2</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>8</td>
</tr>
<tr>
<td>Linezolid</td>
<td>4</td>
</tr>
<tr>
<td>Minocycline</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Penicillin</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Pip/Tazo</td>
<td>8</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>2</td>
</tr>
</tbody>
</table>

**All Gram neg**

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>Tigecycline %S MIC&lt;sub&gt;90&lt;/sub&gt; %S MIC&lt;sub&gt;90&lt;/sub&gt; %S MIC&lt;sub&gt;90&lt;/sub&gt; %S MIC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>E. coli/Kleb</td>
<td>8</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>8</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Imipenem</td>
<td>2</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Minocycline</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Pip/Tazo</td>
<td>64</td>
</tr>
</tbody>
</table>
Antimicrobial susceptibility of tigecycline

Results: The tables summarise results for all isolates, and for specific key pathogens.

Conclusions: Tigecycline’s consistently low MIC90 values and broad spectrum of activity, including otherwise resistant strains, should make it a useful option for difficult-to-treat infections.

**P1597** In vitro antibacterial activity of Tigecycline against multidrug resistant Acinetobacter baumannii isolates

N. Iris, T. Yildirimak, M. Erozs Arat*, F. Simsek (Istanbul, TR)

Objectives: Acinetobacter baumannii is known to be highly resistant in hospital settings and has always been a challenge for clinicians and hospital infection control. We performed this study to investigate Tigecycline susceptibility of multidrug resistant A. baumannii isolates which have been found in patients infected by these bacteria in our hospital.

Methods: Between January and December 2008, 60 multidrug resistant Acinetobacter baumannii isolates were recovered from inpatients of Okmeydanı̈ Teaching and Research Hospital. Tigecycline susceptibility was determined by disc-diffusion method and E test; other antibiotic susceptibilities and identification were performed by mini-API automated identification and susceptibility system.

Results: The isolates were recovered from 29 endotracheal aspirates (48.4%), 11 wounds (18.3%), 11 haemocultures (18.3%), 3 urines (5%), 3 sputums (5%) and 3 spinal fluids (5%). The majority of the samples were taken from inpatients of intensive care units. 59 isolates (98.3%) were susceptible to Tigecycline, only one isolate was resistant to Tigecycline by E test method (MIC = 16).

Conclusion: Tigecycline may be an effective and reliable therapeutic option against strains of Acinetobacter baumannii, including multidrug resistant strains.

**P1598** In vitro activity of tigecycline and other commonly used antibiotics against Burkholderia cepacia complex and other multi-resistant, difficult-to-treat cystic fibrosis-associated Gram-negative non-fermentative bacteria

J. Kenning, M. Denton* (Leeds, UK)

Objectives: To ascertain the in vitro activity of tigecycline and other commonly used antibiotics against Burkholderia cepacia complex (Bcc) and other multi-resistant, difficult-to-treat cystic fibrosis (CF)-associated Gram negative non-fermentative bacteria.

Methods: The test panel contained 157 non-duplicate isolates collected from respiratory samples of people with CF (143 isolates) or laboratory controls (14), and comprised of Burkholderia multivorans (BM) (37 isolates), B. cenocepacia (BC) (23), other Bcc members (12), Stenotrophomonas maltophilia (SM) (49), Achromobacter xylosoxidans (AX) (20) and other CF-associated species (16). MICs of tigecycline and 10 other commonly used antibiotics were determined using Etest. BSAC breakpoints (or CLSI if not available) were used to determine susceptibility. Synergy testing with tigecycline in combination with one of eight other antibiotics was performed using an Etest method. Synergy was defined as a summation fractional inhibitory concentration of <0.5.

Results: Tigecycline exhibited good activity against AX and SM, with 85% and 78% of isolates fully susceptible, respectively. All tested isolates of Pandoraea spp and Raistonia spp were fully susceptible. Activity against different members of the Bcc was variable with only 13% of BC, 3% of BM, and 33% of other members being fully susceptible. By comparison, 91% of BC, 97% of BM and 92% of other Bcc members were fully susceptible to minocycline. Antagonism between tigecycline and other agents was rarely encountered, except when used in combination with colistin. However, the occurrence of synergy was variable. The most synergistic combination against members of the Bcc was with ceftazidime, with enhancement of activity against 17% of BC and 19% of BM. Synergy with meropenem was less common with enhanced activity occurring against 0% of BC and 14% of BM.

Conclusions: These data suggest tigecycline has useful activity against some multi-resistant, difficult-to-treat pathogens in people with CF, and could be used as an alternative to an aminoglycoside when combined with a β-lactam. Clinical studies are needed to ascertain the correlation between in vitro susceptibility, synergy testing and patient outcomes.
The Tigecycline Evaluation Surveillance Trial (T.E.S.T.) program is an ongoing global surveillance with the first post-marketing prospective report of tigecycline and comparator in vitro activity for the years 2004 through 2008. This study evaluates trends in susceptibility of Acinetobacter spp. and Pseudomonas aeruginosa isolated in Europe during this time period.

Methods: More than 4,530 clinical isolates were collected from 78 investigative sites in 22 countries in Europe. Clinical isolates were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to EUCAST guidelines.

Results: Summary data for tigecycline and comparators by year are presented in the table.

Conclusions: Tigecycline demonstrated no shift in MIC values in Europe over four years from its pre-marketing baseline values when tested against non-fermenters. Consistent MIC90 values (1 mcg/mL) against Acinetobacter spp., including strains resistant to other drugs, may make it an option when treating infections caused by strains resistant to treatment with other agents.

### Table: Changes in susceptibility of select nonfermenters in Europe: 2004–2008

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acinetobacter spp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tigecycline MIC90</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tigecycline MIC90</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>
**Background:** ESBL producing organisms continue to be a therapeutic dilemma for physicians as many currently marketed antimicrobials are ineffective against these strains. Carbapenems and newer antimicrobials such as tigecycline have proven to effective against many of these strains. Tigecycline, the first member of the glycylcyclines, was marketed in mid 2005 and has demonstrated success against ESBL producing organisms. Four years of data are now available on the incidence and activity of tigecycline against these strains.

**Methods:** All clinical isolates identified as *E. coli, K. pneumoniae* and *K. oxytoca* were confirmed as ESBL producers or non-ESBL producers using criteria established by CLSI. MICs were determined by broth microdilution according to CLSI guidelines using identical panels.

**Results:** Results of trends in incidence and antimicrobial susceptibility for tigecycline for ESBLs for each country between 2004 and 2008 are listed in the table.

<table>
<thead>
<tr>
<th>Country</th>
<th>2004 %ESBL</th>
<th>2005 %ESBL</th>
<th>2006 %ESBL</th>
<th>2007 %ESBL</th>
<th>2008 %ESBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>0 NA</td>
<td>1.46 1</td>
<td>3.1 4</td>
<td>2.9 1</td>
<td>3.0 1</td>
</tr>
<tr>
<td>China</td>
<td>18 1</td>
<td>34.8 8</td>
<td>24.1 0.25</td>
<td>NA NA NA</td>
<td>NA NA NA</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>NA NA</td>
<td>0 NA 0</td>
<td>NA 7.2 0.5</td>
<td>NA NA NA</td>
<td>NA NA NA</td>
</tr>
<tr>
<td>India</td>
<td>65.3 1</td>
<td>NA NA</td>
<td>0 NA 12.6</td>
<td>1 10.5 1</td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>NA NA</td>
<td>26.5 6 2</td>
<td>18.5 24.1</td>
<td>1 1</td>
<td></td>
</tr>
<tr>
<td>Pakistan</td>
<td>69.2 4</td>
<td>17.6 2</td>
<td>0 NA NA NA</td>
<td>NA NA NA</td>
<td></td>
</tr>
<tr>
<td>Philippines</td>
<td>2.1 0.5</td>
<td>NA NA 9.8</td>
<td>2 9.1 0.5</td>
<td>8.8 0.5</td>
<td></td>
</tr>
<tr>
<td>Singapore</td>
<td>18.4 1</td>
<td>0 NA 27.1</td>
<td>0.5 9.2 2</td>
<td>NA NA NA</td>
<td></td>
</tr>
<tr>
<td>Taiwan</td>
<td>NA NA</td>
<td>NA NA 18.3</td>
<td>2 24.1 2</td>
<td>22.2 2</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:** With exception of Taiwan it would appear the rates of ESBLs producing organisms are going down. High rates still exist in India, Korea and Taiwan. Tigecycline in vitro activity varies by country.

**Activity of tigecycline and five comparators against recent Gram-positive anaerobes in Europe – the Tigecycline European Surveillance Trial**

**Methods:** 454 Gram-positive anaerobic pathogens were collected and identified from 18 sites in 6 countries in Europe. MICs of tigecycline and five comparators were determined per EUCAST guidelines using agar dilution.

**Results:** European Gram-positive pathogens tested against tigecycline and comparators are shown in the table.

**Conclusions:** Tigecycline showed excellent in vitro activity against European clinical isolates of *Clostridium difficile*, with MIC50/MIC90 values of <0.06 mcg/mL. Tigecycline's low MIC50/MIC90 values suggest that it may be an option against this difficult to treat pathogen.

**In vitro susceptibility of tigecycline and to an extended panel of antimicrobials of Gram-positive and Gram-negative isolates in Portugal**

**Objectives:** Tigecycline is a new antibiotic active against a variety of bacterial species, including strains resistant to other antibiotics. In order to increase awareness of this antibiotic in Portuguese hospitals we determined the susceptibility to tigecycline and to several other

Methods: A total of 1485 clinically significant isolates were collected and identified at 16 hospitals in Portugal. Susceptibilities were determined at the coordinating laboratory by disk diffusion. The antimicrobials tested against Gram-positive bacteria were: tigecycline, vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, levofloxacin, gentamicin, rifampicin, tetracycline, clindamycin, ampicillin, streptomycin, tetracycline, penicillin, cefotaxime (the latter were also tested using E-test for S. pneumoniae). The antimicrobials tested against Gram-negative bacteria were: tigecycline, levofloxacin, gentamicin, imipenem, pipercillin-tazobactam, cefepime, ceftazidime, ampicillin-sulbactam, trimetoprim-sulfamethoxazole, minocycline, amoxicillin-clavulanate, cefotaxime, gentamicin, amikacin, ampicillin, ciprofloxacin and tetracycline.

Results: Among Gram positive isolates (n = 839), all Staphylococcus spp. (n = 388) were fully susceptible to tigecycline, including methicillin-resistant strains. All Enterococcus spp. (n = 160) isolates, including vancomycin-resistant strains were susceptible to tigecycline, as well as all the Streptococcus spp. isolates tested (n = 291).

Among Gram negative isolates (n = 646), ESBL-producing or quinolone-resistant Escherichia coli strains (n = 252) were susceptible to tigecycline, while approximately 90% of ESBL-producing or quinolone-resistant Klebsiella spp. strains (n = 134) were susceptible to this antibiotic. Among Haemophilus influenzae (n = 53), Enterobacter spp. (n = 80), Stenotrophomonas maltophilia (n = 67), and Acinetobacter baumannii (n = 80) we found susceptibilities to tigecycline of 100%, 96%, 87% and 31%, respectively.

Conclusion: Tigecycline showed a good activity against most of the pathogens included in this study, including strains resistant to many broad-spectrum antibiotics, showing that tigecycline could be a valid choice for difficult to treat infections.

**P1607** Profile of tigecycline and other agents against enteric bacilli collected across Europe


Objective: Tigecycline (TIG), a derivative of minocycline, has a broad spectrum of activity which includes Enterobacteriaceae (EN). EN infections can be problematic to treat due to antibiotic resistance (R). Monitoring the activity of TIG against EN with clinically relevant R and for the continued emergence of R among EN is important. This study examines the activity of TIG and comparators against EN across Europe (EU).

Methods: In total, 1,307 EN (464 E. coli [EC], 397 K. pneumoniae [KP], 146 Citrobacter spp. [CS], 189 Enterobacter spp. [ES], and 111 S. marcescens [SM]) isolates were obtained from Belgium, Czech Republic, France, Germany, Hungary, Italy, Spain, and the United Kingdom during 2006–08. Isolates were centrally tested by broth microdilution (CLSI M7-A7). EUCAST breakpoints (BP) were used to interpret TIG MIC results and CLSI (M100-S18) BP were used to interpret all other agents, where applicable.

Results: TIG MIC90 was 1 mg/L against EN overall and % susceptibility (S) was 96.4, comparable to imipenem (MIC90 of 1 mg/L; 99.8% S). Results: Tigecycline demonstrated broad antimicrobial activity against common pathogens associate with numerous types of clinical infections occurring in EU. Tigecycline was active against antimicrobial-resistant strains including MRSA, VRE, multidrug-resistant isolates of Enterobacteriaceae, including those producing broad-spectrum β-lactamases as ESBLs. Based on the potency and spectrum of tigecycline shown here for 2008 isolates, this agent has a role in empiric therapy for treating bacterial pathogens in these EU nations.

Conclusions: Tigecycline demonstrated broad antimicrobial activity against common pathogens associated with numerous types of clinical infections occurring in EU. Tigecycline was active against antimicrobial-resistant strains including MRSA, VRE, multidrug-resistant isolates of Enterobacteriaceae, including those producing broad-spectrum β-lactamases as ESBLs. Based on the potency and spectrum of tigecycline shown here for 2008 isolates, this agent has a role in empiric therapy for treating bacterial pathogens in these EU nations.

**P1609** Worldwide activity of tigecycline against community-acquired respiratory tract infection pathogens collected during 2006–2008

D. Biedenbach, R. Jones, P. Strahula, H. Sader* (North Liberty, US)

Objectives: To assess the contemporary potency and spectrum of tigecycline against Gram-positive and -negative CARTI pathogens,
including strains with various resistant (R) phenotypes. Tigecycline is a glycyclcline antimicrobial with proven activity against numerous bacterial species, including staphylococci and streptococci. Based upon in vitro activity and demonstrated clinical trial efficacy, tigecycline has received approval in the USA and Europe (EU) for treating complicated skin and skin structure and intra-abdominal infections.

**Methods:** A total of 9,235 clinically-significant non-duplicate isolates from patients with CARTI were collected from North and Latin America and EU medical centres participating in surveillance of tigecycline (2006–2008). Susceptibility (S) testing was performed by a central laboratory (JMI Laboratories) using CLSI methods (M7-A7, 2006) and all quality control tests were within published ranges.

**Results:** Tigecycline was active against all (≤2 mg/L) tested strains as summarised in the Table.

<table>
<thead>
<tr>
<th>Organism (no. tested)</th>
<th>TIGMIC (mcg/mL)</th>
<th>Curr. % inhibited at Tigecycline MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S%</td>
<td>S%</td>
</tr>
<tr>
<td>S. aureus (174)</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Oxacillin-R (809)</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Oxacillin-S (264)</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>S. pneumoniae (5,377)</td>
<td>≤0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Penicillin-R (5,159)</td>
<td>≤0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Penicillin-S (881)</td>
<td>≤0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Penicillin-2% (977)</td>
<td>≤0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>H. influenzae (3,129)</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>[β-lactamase- (2,482)]</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>M. catarrhalis (17)</td>
<td>0.12</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Tigecycline inhibited *S. aureus* at ≤0.5 mg/L, with a MIC90 of 0.25 mg/L, regardless of S or R to oxacillin. Tigecycline also had good activity against *S. pneumoniae* isolates (MIC90, 0.06 mg/L), including penicillin-R strains. The MIC90 for fastidious Gram-negative pathogens was 1 mg/L for *H. influenzae* (HI) and 0.12 mg/L for *M. catarrhals* (MCAT). There was no significant difference in tigecycline activity between the three monitored regions for any of CARTI pathogens.

**Conclusions:** Tigecycline demonstrated broad antimicrobial activity against pathogens associated with CARTI. Tigecycline was active against antimicrobial-R strains including oxacillin-R staphylococci and *S. pneumoniae*, as well as HI and MCAT isolates, including those producing β-lactamase enzymes. Tigecycline potency and spectrum shown here for 2006–2008 confirms that this agent may have a role in treating CARTI.

**P1610** Tigecycline in vitro activity against isolates of *S. pneumoniae* and *H. influenzae*: a multi-year update from the T.E.S.T. Program in 2008


**Background:** Tigecycline (TIG), a new glycyclcline, has been shown to have potent broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the in vitro activity of TIG compared to 10 comparators against methicillin-resistant *S. aureus*, 140 *S. pneumoniae* isolates from more than 30 countries from 2004 to 2008.

**Methods:** A total of 5,084 clinically significant respiratory isolates collected worldwide were analyzed in this study. The isolates were identified to the species level at the participating sites and confirmed by the central laboratory. MICs were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines.

**Results:** Activities of tigecycline and comparator antimicrobials are shown in the table. Overall, 23.3% of *H. influenzae* were β-lactamase producers and 38.1% of *S. pneumoniae* presented some degree of non-susceptibility to penicillin. Tigecycline demonstrated potent inhibitory activity with an MIC90 of ≤0.5 mcg/ml against β-lactamase positive *H. influenzae* and penicillin non-susceptible *S. pneumoniae*.

**Conclusions:** Tigecycline showed excellent inhibitory activity against *H. influenzae* and *S. pneumoniae* regardless of the presence of β-lactamase or penicillin-resistance mechanisms. The results of this study suggest that tigecycline may be a reliable therapeutic option for the treatment of respiratory infections due to these species.


**Background:** Tigecycline (TIG), a member of a new class of antimicrobials (glycyclclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the in vitro activity of TIG compared to amoxicillin-clavulanic acid, piperacillin-tazobactam, levofloxacin, ceftriaxone, linezolid (LZD), minocycline (MIN), vancomycin (VAN), ampicillin, penicillin, and imipenem (IMP) against methicillin-resistant *S. aureus* (MRSA) isolates collected from 316 sites in 24 European countries between 2004 and 2008.

**Methods:** A total of 1016 clinical isolates of MRSA were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentration (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to EUCAST guidelines.

**Results:** The %S for the study drugs with MRSA activity–TIG, VAN, LZD, and MIN–was 99.9, 100, and 83.7, respectively. MIC90/90 (mcg/ml) for TIG, VAN, LZD, and MIN were 0.12/0.25, 1/1, 2/4, and ≤0.25/4, respectively.

**Conclusions:** European susceptibility patterns of MRSA remain fairly consistent. TIG was as potent as VAN and LZD, inhibiting 1015/1016 (99.9%) of the MRSA isolates at their respective breakpoints. TIG’s excellent expanded broad spectrum of activity against MRSA should make it a very useful drug in treatment of difficult staphylococcal infections.

**P1612** Comparative in vitro activity of tigecycline against pathogens obtained from intensive care patients in Germany

R. Matters, W.R. Heizmann, B. Körber-Irrgang, E. Leitner, M. Kresken* (Marburg, Berlin, Rheinbach, Munster, DE)

**Objectives:** Tigecycline has been shown to be active against a wide range of bacteria including multi-resistant pathogens. The German TIG Evaluation Trial (G-TTEST) is an ongoing surveillance programme comprising 15 German laboratories monitoring the susceptibility of bacterial pathogens to tigecycline. The objective of this study was to evaluate the in vitro activity of tigecycline against intensive care unit isolates of four Gram-positive and three Gram-negative species.

**Methods:** A total of 996 isolates (152 *S. aureus* [of which 83 were MRSA], 126 *S. epidermidis*, 96 *E. faecalis*, 140 *E. faecium*, 182 *E. cloacae*, 179 *E. coli*, 121 *K. pneumoniae*) collected in...
Profile of tigecycline and comparator agents against Gram-positive organisms collected across the United States and Europe in 2007–2008

N. Brown, C. Pillar*, D. Dragoi, C. Thornberry, D. Sahn, M. Dowzicky (Chantilly, Collegeville, US)

Objective: Tigecycline (TIG), a broad-spectrum glycylcycline, has been shown to have broad spectrum of activity against most bacterial pathogens. The T.E.S.T program determined the in vitro activity of tigecycline and 10 comparators against aerobic Gram-positive and negative bacterial species.

Methods: A total of 200 non-duplicate clinical isolates collected in 2006 were analyzed in this survey. Minimum inhibitory concentrations (MICs) were determined using supplied broth microdilution panels and interpreted according to CLSI guidelines. Breakpoints defined by EUCAST were used for tigecycline, where applicable.

Results: TIG showed excellent inhibitory activity against both Gram-positive and Gram-negative bacteria (except for Streptococcus agalactiae and Pseudomonas aeruginosa), representing an effective option for treatment of infections caused by these pathogens.

Conclusion: Tigecycline showed excellent inhibitory activity against both Gram-positive and Gram-negative bacteria (except for Streptococcus agalactiae and Pseudomonas aeruginosa), representing an effective option for treatment of infections caused by these pathogens.
**Antibacterial susceptibility of Gram-positives**

**P1616** Activity of tigecycline against multidrug-resistant anaerobic isolates from Europe in 2008 – the T.E.S.T. Program


Background: Tigecycline, a member of a new class of antimicrobials (glycyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the in vitro activity of tigecycline compared to piperacillin-tazobactam, clindamycin, metronidazole, meropenem, penicillin, and cefoxitin against anaerobic multi-drug resistant (MDR) strains collected from 18 investigational sites in 6 countries in Europe throughout 2008.

**Methods:** A total of 1090 clinical anaerobic pathogens were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined using agar dilution. Antimicrobial resistance was interpreted according to EUCAST breakpoints or CLSI and FDA breakpoints where no EUCAST breakpoints were available. Strains were grouped by resistance to 0, 1, or 2 drug classes.

**Results:** The MIC90s of tigecycline to MDR groups 0–2 are shown in the table.

<table>
<thead>
<tr>
<th>MDR Group</th>
<th>MIC90 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 0</td>
<td>0.12 (33)</td>
</tr>
<tr>
<td>Group 1</td>
<td>0.12 (10)</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.12 (12)</td>
</tr>
</tbody>
</table>

**Conclusions:** Tigecycline retained activity against 100% of multi-drug resistant anaerobes, with MIC90 values ≤ 4 mcg/mL. Tigecycline's in vitro activity against multi-drug resistant anaerobic pathogens should prove useful in the treatment of infections caused by such therapeutically challenging strains.
Notably, ORI demonstrated potent in vitro activity against both pan-sensitive isolates and those resistant to VAN, DAP, or LIN.

<table>
<thead>
<tr>
<th>Organism or phenotype</th>
<th>ORI MIC&lt;sub&gt;50&lt;/sub&gt; (%S)</th>
<th>VAN MIC&lt;sub&gt;50&lt;/sub&gt; (%S)</th>
<th>DAP MIC&lt;sub&gt;50&lt;/sub&gt; (%S)</th>
<th>LIN MIC&lt;sub&gt;50&lt;/sub&gt; (%S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae (125)</td>
<td>0.015 na</td>
<td>0.05 na</td>
<td>0.5 na</td>
<td>2 na</td>
</tr>
<tr>
<td>Penicillin-RES (46)</td>
<td>0.009 na</td>
<td>0.05 na</td>
<td>0.5 na</td>
<td>2 na</td>
</tr>
<tr>
<td>Macrolide-RES (64)</td>
<td>0.008 na</td>
<td>0.05 na</td>
<td>0.5 na</td>
<td>2 na</td>
</tr>
<tr>
<td>S. agalactiae (127)</td>
<td>0.25 na</td>
<td>1 0.05 0.5 0.25 0.1 0.2 1 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. pyogenes (118)</td>
<td>0.25 na</td>
<td>0.5 0.25 1 0.25 0.1 0.25 0.1 0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viridans group strep (104)</td>
<td>0.06 na 1 0.9 1 0.9 1 0.6 2 1 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*na = breakpoints not defined.

**Activity of telavancin against reference and recent clinical isolates of hVISA and VISA assessed by population analysis profiling**


**Objectives:** Infections caused by vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogeneous VISA (hVISA) are associated with high rates of vancomycin (VAN) treatment failure. Telavancin (TLV) possesses potent activity against *S. aureus*, including methicillin-resistant *S. aureus* (MRSA), hVISA and VISA isolates. We used population analysis profiling (PAP) to evaluate the activity of TLV against hVISA and VISA isolates.

**Methods:** A total of 16 recent clinical *S. aureus* isolates and 4 reference strains including 1 vancomycin-susceptible *S. aureus* (VSSA), 1 hVISA, and 2 VISA were tested. Minimum inhibitory concentrations (MICs) were determined by broth microdilution (CLSI). Screening methods to identify hVISA included a modified VAN agar screen (Brain Heart Infusion agar containing 3 mg/L VAN [VAN-3], VAN and teicoplanin (TEI) Macro Etest and VAN PAP). VAN PAP-area under the plasma concentration-time curve (AUC) ratios (AUC of test strain/AUC of Mu3) were used to categorise VSSA (<0.9), hVISA (0.9–1.3) and VISA (>1.3). TLV PAP assays were conducted on selected isolates.

**Results:** MICs for all 16 clinical isolates were 0.25–1 mg/L for TLV and 1–4 mg/L for VAN. Three isolates with VAN MIC=4 mg/L were classified as VISA. The remaining 13 isolates were screened for the hVISA phenotype; 6 grew on VAN-3 agar, 4 of these had VAN MIC=2 mg/L, and, of these, 3 isolates were identified by Macro Etest as potential hVISA. VAN PAP analysis of the 4 isolates with VAN MIC=2 mg/L identified 2 as hVISA (VAN PAP AUC ratios of 0.9 and 1.0), 1 as VISA (ratio of 1.7) and 1 as VSSA (ratio of 0.8). TLV PAP analysis of these 4 isolates and the 4 reference strains revealed no TLV heteroresistant subpopulations. Against all tested strains, no colony growth was observed in TLV PAP assays at concentrations >0.5 mg/L.

**Conclusions:** This report summarises the first attempt to identify potential TLV heteroresistance in *S. aureus*. PAP studies demonstrated that TLV was uniformly active against all VSSA, hVISA and VISA isolates. These results are consistent with the improved targeting of Lipid II by TLV relative to VAN. No evidence for telavancin heteroresistance was detected.
1 to 2 dilutions lower than those of vancomycin, as measured by the agar dilution method. The MIC 50 and MIC90 of telavancin for all isolates were 0.25 \( (\text{range,} <0.125 \text{ to } 0.5 \text{ mg/L}) \) and 0.5 mg/L \( (\text{range,} <0.125 \text{ to } 1.0 \text{ mg/L}) \) respectively, whereas the overall MIC 50 and MIC 90 for vancomycin by the agar dilution method were 1.0 \( (\text{range} \ 0.5 \text{ to } 1.0 \text{ mg/L}) \) and 2.0 mg/L \( (\text{range} \ 0.5 \text{ to } 2.0 \text{ mg/L}) \) Not all MICs obtained on the Vitek\textsuperscript{2} system could be compared as 375 strains had an MIC to vancomycin of <1.0 mg/L and 8 strains failed to grow, making exact MIC values unavailable. For 286 strains exact MIC values of vancomycin by agar dilution and Vitek\textsuperscript{2} were available. MIC of all but three of 204/286 (71.3\%) strains with non-concordant results was at least 1 dilution higher by the Vitek\textsuperscript{2} system.

**Conclusion:** Telavancin is more active against CoNS than vancomycin, with MICs 1 to 2 dilutions lower. For evaluable isolates the Vitek\textsuperscript{2} system tended to report MICs of vancomycin one dilution higher than agar dilution.

**P1622 In vitro profiling of the activity of ceftaroline against European clinical isolates collected in the CANVAS 1 and 2 trials of complicated skin and skin-structure infections**


**Objectives:** Ceftaroline (CPT) is an investigational, parenteral, cephalosporin exhibiting activity against Gram-positive organisms, including methicillin resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant *Streptococcus pneumoniae*, as well as Gram-negative organisms. CPT has recently been evaluated in patients with complicated skin and skin structure infections (cSSSI) in two blinded multinational Phase 3 trials (CANVAS 1 and 2). The aims of this paper were to determine the national distribution of pathogens collected from European subjects enrolled in CANVAS 1 and 2; to assess the prevalence of key resistance phenotypes; and to determine the activity of CPT against baseline pathogens.

**Methods:** From the CANVAS 1 and 2 trials, a total of 840 isolates from subjects with cSSSI were collected at sites in Austria, Germany, Latvia, Poland, Romania, Russia, United Kingdom and Ukraine. All isolates were centrally tested for susceptibility to CPT and other agents using broth microdilution in accordance with CLSI guidelines.

**Results:** In Europe (EU), the most commonly isolated skin pathogens were *Staphylococcus aureus* (SA) and *Streptococcus pyogenes* (SP) with prevalence rates of 49\% and 12\%, respectively. Among SA, the majority of isolates were collected from Russia (51\%), followed by Ukraine (15\%), Romania (10\%) and Poland (10\%). The prevalence of methicillin resistance among SA isolates varied by country, and ranged from 0\% in Austria and Latvia to 20\% in Russia and 35\% in Romania. CPT was active in vitro against SA, regardless of geographic origin, with MIC\textsubscript{90} values of 0.25 or 0.5 mg/L for each country. CPT was active against both methicillin-susceptible SA and MRSA (MIC\textsubscript{90} of 0.25 and 1 mg/L, respectively). All SP and other \( \beta \)-haemolytic streptococci (BHS) were inhibited by CPT at \( \leq 0.015 \text{ mg/L} \). CPT was also active against *Enterococcus faecalis* and *Escherichia coli* with MIC\textsubscript{90} of 1 and 0.5 mg/L, respectively. CPT was less active against *Klebsiella pneumoniae* (MIC\textsubscript{90}, >16 mg/L), with 11\% of isolates resistant to ceftazidime (CAZ).

**Conclusions:** A diverse collection of skin pathogens was collected from European subjects during CANVAS 1 and 2. While MRSA rates varied by country, CPT retained in vitro activity against all SA isolates regardless of geographic origin. CPT was also active against BHS and most CAZ-susceptible Gram-negative enteric bacilli. CPT exhibits potent in vitro activity against isolates from subjects with cSSSI.
Daptomycin susceptibility of Gram-positive pathogens

In vitro activity of daptomycin combined with other antimicrobial agents against Gram-positive cocci

**Objectives:** Daptomycin (DAP), a cyclic lipopeptide, exerts rapid bactericidal activity against clinically important Gram-positive bacteria including multidrug resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Since DAP may be used in combination with other antibiotics, we evaluated the in vitro activities of DAP in combination with 14 other drugs against a panel of *S. aureus* (SA) and enterococcal isolates.

**Methods:** Thirty strains including VRE, MRSA, vancomycin-intermediate SA and daptomycin-resistant isolates were studied. Synergy testing was performed by using the checkerboard broth microdilution method. DAP in combination with VAN, GEN, fosfomycin (FOS), piperacillin-tazobactam (P/T), amikacin (AMK), rifampin (RIF), cefazidine (CAZ), ceftriaxone (CRO), meropenem (MEM), imipenem (IMP), ciprofloxacin (CIP), moxifloxacin (MOX), clindamycin (CLI) were tested against 10 SA strains (in total 140 drug combination tests), while DAP in combination with VAN, GEN, AMK, RIF, or ampicillin (AMP) was tested against 10 strains each of *Enterococcus faecalis* (EFM) and *E. faecium* (EFS) (in total 140 drug combination tests) per strain.

**Results:** Of 140 drug combination tests performed with the 10 SA strains, 125 showed no significant effect. In contrast, synergy was observed for 6, 3, 4, and 2 strains with CAZ, CRO, P/T and IMP, respectively. Likewise, the vast majority of drug combination tests performed with the 10 EFS strains revealed indifferent effects. However, DAP plus RIF was synergistic against one strain. Of the 50 drug combination tests performed with the 10 EFM strains, 31 showed no significant effect, while synergy was observed for 7, 6, 3, and 2 strains with RIF; AMP, GEN, and VAN, and for one strain with AMK.

**Conclusion:** Generally, DAP combinations showed no significant synergistic effects, however with β-lactams (in particular CAZ) there may be a synergistic effect against some SA strains, while DAP combined with rifampin or ampicillin may be useful in the treatment of EFM infections.

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**P1625 Daptomycin susceptibility of Gram-positive pathogens collected from 61 centres in 9 European countries in 2007**

I. Morrissey* on behalf of the Dutch Rapid MRSA Diagnostics Study Group

**Objectives:** Daptomycin (DAP) has been shown to be highly active against contemporary Gram-positive bacteria (GBP) circulating in several European countries (Daptomycin Susceptibility of Contemporary Gram-positive pathogens circulating in Europe between 2004 and 2007. CLSI broth microdilution MIC was determined for DAP and selected comparators. EUCAST and/or CLSI breakpoints were used where available.

**Methods:** 2,305 GBP: *Staphylococcus aureus* (SA), coagulase-negative staphylococci (CNS), *Enterococcus faecalis* & *E. faecium*, β-haemolytic streptococci (BHS), ‘viridans’ streptococci (VS) and *Corynebacterium* spp. (COR) were collected from the Czech Republic, Denmark, Greece, Hungary, Norway, Poland, Russia, the Slovak Republic and Turkey during 2007. CLSI broth microdilution MIC was determined for DAP and selected comparators. EUCAST and/or CLSI breakpoints were used where available.

**Results:** All SA, 99.8% of CNS, 99.8% of BHS and all COR were inhibited by ≤1 mg/L DAP and 99.8% of enterococci were inhibited by ≤4 mg/L DAP VS were slightly less susceptible to DAP (90.5%) using the break point of 1 mg/L. Most of the isolates that were non-susceptible to DAP had a MIC generally only one dilution above the break point for susceptibility. Linezolid, vancomycin and tigecycline were also active with 99.8%, 99.5% and 95.4% of isolates being susceptible to these agents. DAP susceptibility of isolates collected in the 9 countries of this study were similar to that seen in other countries (1).

**Conclusion:** DAP was very active against contemporary GBP in the 9 European countries studied and susceptibility in these countries was similar to that seen in other European countries. DAP remains an excellent alternative therapeutic option for the treatment of infections caused by GBP.

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**P1626 Daptomycin susceptibility of contemporary Gram-positive pathogens circulating in Europe between 2004 and 2007**

I. Morrissey* on behalf of the European Daptomycin Study Group

**Objectives:** Daptomycin (DAP) is a novel cyclic lipopeptide antibiotic active against Gram-positive bacteria (GBP) and has been available for use in Europe since early 2006 for the treatment of Gram-positive infections. Monitoring susceptibility of pathogens to new agents is necessary to predict or detect possible development of resistance that could ultimately undermine its utility. The objective of this study was to compare the susceptibility of selected Gram positive pathogens circulating in 12 European countries in 2004–5 with that of isolates circulating in the same centres in 2007.

**Methods:** In late 2004 and early 2005, 2867 GBP [Staphylococcus aureus (SA), coagulase-negative staphylococci (CNS), *Enterococcus faecalis* & *E. faecium*, β-haemolytic streptococci, ‘viridans’ streptococci (VS) and *Corynebacterium* spp. (COR)] were collected from Austria, Belgium, France, Germany, Ireland, Italy, Netherlands, Portugal, Spain, Sweden, Switzerland and UK. In 2007, 2973 GBP were collected from the same centres. CLSI broth microdilution MIC was determined for DAP, vancomycin (VAN), linezolid (LZD) and other comparators. EUCAST and/or CLSI breakpoints were used where available.

**Results:** DAP was highly active with 99.7% of the 5,840 isolates collected being susceptible. Activity against staphylococci and enterococci was unaffected by their susceptibility to methicillin and vancomycin respectively. Susceptibility of isolates collected during 2007 was very similar to that of isolates collected during 2004–5. Few (0.3%) of isolates were non-susceptible to DAP and for most of these, the DAP MIC was only one dilution above the break point for susceptibility. VAN and LZD were also highly active with >99% of isolates being susceptible. LZD-resistant enterococci increased from 0.2% in 2004–5 to 4.5% in 2007. No differences in VAN susceptibility were seen between the two collection periods.

**Conclusion:** DAP, VAN and LZD were very active against contemporary GBP in Europe. There was no evidence for an increase in DAP or VAN NS in currently circulating GBP but LZD resistant enterococci were more prevalent in 2007 than in 2004–5. DAP remains an excellent alternative therapeutic option for the treatment of infections caused by GBP.
Royal jelly's effect on glucosyltransferase expression in *Staphylococcus aureus*

A. Shoae Hassani*, K. Hamdi (Fars, Tehran, IR)

**Objectives:** The bulk of dental plaque is composed of bacterial derived extracellular polysaccharide known as glucan, which is synthesized by streptococcal glucosyltransferase (Gtf) enzymes. The objective of this study was to evaluate the specific efficacy of Royal Jelly Extract (RJE) on Glucosyltransferase (the biofilm initiation involved enzyme in *Streptococcus mutans*) that is responsible for dental caries and bacteraemias following dental manipulations. Royal Jelly so called because it is the exclusive food of the Queen bees, the creamy product secreted by young nurse worker bees (Apis mellifera) to feed the queen and queen larvae.

**Methods:** Royal jelly was supplied by a traditional Bee keeper in Lahijan, a city situated in the south coast of Caspian Sea, north of Persia. The sample was obtained in spring (April) and was transferred to the Science and Research campus of IAU in Tehran, in the ice flask. Either soluble and non soluble fractions of the Royal jelly were extracted by means of Clevenger extractor. GC analyses were performed with a Shimadzu 17A gas chromatograph (Shimadzu, Japan) equipped with a flame ionisation detector and a 60 m × 0.25 mm (I.D.) DB-WAX (J&W Scientific, Folsom, CA) fused-silica capillary column. Minimum inhibitory concentration (MIC) of the RJE was assessed by broth dilution method. Examination of the cell adherence (Biofilm inhibitory concentration) was calculated by colony counts from surface scratching of glass slides that were located in the media cultures. Glucosyltransferase expression was detected on 15% SDS poly acrylamide gel electrophoresis and was confirmed by western blot analysis. The ANOVA test (p < 0.05) was used in this study as the statistical method.

**Results:** Concentration of 1 mg ml⁻¹ of RJE was biofilm inhibitor and it was repressed the production of glucosyltransferase completely. Activity of 6 mg ml⁻¹ of RJE was bacteriostatic and 30 mg ml⁻¹ of RJE was bactericidal for *S. mutans* (p < 0.05).

**Conclusion:** The results of the assays suggest that RJE was able to block the major enzyme that contributes to *S. mutans* biofilm formation in low concentrations in vitro. The growth of *S. mutans* was prevented completely in higher concentrations of RJE.

**Antimicrobial resistance of *Staphylococcus aureus* in a university hospital, Setif, Algeria, from 2002–2006**

F. Sahli*, N. Radji, A. Touabi (Setif, DZ)

**Objectives:** To assess the antimicrobials resistance (R) of *Staphylococcus aureus* (SA) in the university hospital of Setif, Algeria from 2002–2006 among inpatients (IN) and outpatients (OUT).

**Methods:** 1342 SA strains isolated between 2002 and 2006 were studied for their susceptibility to oxacillin (OXa), gentamicin (GEN), kanamycin (KA), amikacine (AK), erythromycin (ERM), pristinamycin (PRI), oxacillin (OEx), fusidic acid (FA), fosfomycin (FO), rifampicin (RIF), and vancomycin (VA) according to CLSI guidelines.

**Results:** OXA R was observed for 29.7% among IN and 25.9% among OUT. For the other antimicrobials, the higher rates of resistance were mentioned for KA and FA: 42.5% for IN, 35.7% for OUT and 38.7% for IN, 40.3% for OUT respectively. 7.3% and 4.2% of IN and OUT strains respectively were resistant to OFX. None strain was resistant to VA. Wounds and skin isolates were resistant in 30.1% to OXA while bacteraemias strains were resistant in 20.2% to the same antimicrobial.

**Conclusion:** IN SA and OUT SA strains had nearly the same OXA R rates. The OXA R strains had the higher resistance rates to KA and FA. The higher resistance rates were observed in wounds and skin isolates.

**Staphylococcus aureus from blood stream infections**

B. Catry, E. Hendrickx*, R. Preal, R. Mertens (Brussels, BE)

**Objectives:** In blood stream infections mortality and treatment costs in hospitals can considerably increase when antimicrobial resistance is present and inappropriate empiric antimicrobial therapy is administered. This study gives an overview of the susceptibility results of *Staphylococcus aureus* isolated from septicemias as reported by diagnostic laboratories in Belgium.

**Methods:** Laboratory results of 397 cases of *Staphylococcus aureus* blood stream infections (BSI) were obtained from 13 voluntary diagnostic laboratories in Belgium during the year 2005 in collaboration with the intermutualistic agency (IMA) coordinating the national health insurance funds. Susceptibility profiles were predominantly based on the Kirby Bauer disk diffusion test according to CLSI guidelines and interpretation was often recorded by semi-automated systems. All participating labs were certified by an external quality control organisation.

**Results:** Only the first isolate per patient during the observation period was included (236 men, 161 women) and the mean age of the patients was 67.5 years (range 0–96). The mean age of patients with methicillin resistant *S. aureus* (MRSA) strains (n = 143) was 70.4 compared to 66.1 when the isolates were susceptible for methicillin (n = 248). The gender distribution for MRSA was 83 men versus 60 women. Susceptibility profiles of the *S. aureus* isolates are presented in the table (resistance percentages include intermediary results).

<table>
<thead>
<tr>
<th>Antimicrobial compound</th>
<th>%R</th>
<th>n tested</th>
<th>(% tested of total S. aureus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>91.57</td>
<td>344</td>
<td>(86.65)</td>
</tr>
<tr>
<td>Ampicillin/amoxicillin</td>
<td>91.98</td>
<td>262</td>
<td>(65.99)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>28.85</td>
<td>104</td>
<td>(26.20)</td>
</tr>
<tr>
<td>Cefalothin (group)</td>
<td>35.71</td>
<td>70</td>
<td>(17.63)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>25.42</td>
<td>59</td>
<td>(14.86)</td>
</tr>
<tr>
<td>Cefotaxim (ceftriaxone)</td>
<td>34.29</td>
<td>70</td>
<td>(17.63)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>32.43</td>
<td>111</td>
<td>(27.96)</td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>37.14</td>
<td>70</td>
<td>(17.63)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>37.08</td>
<td>391</td>
<td>(98.49)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>27.36</td>
<td>106</td>
<td>(26.70)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>38.66</td>
<td>357</td>
<td>(89.92)</td>
</tr>
<tr>
<td>Aztreomycin</td>
<td>30.00</td>
<td>70</td>
<td>(17.63)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>22.73</td>
<td>22</td>
<td>(5.54)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>28.97</td>
<td>390</td>
<td>(98.24)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>15.00</td>
<td>160</td>
<td>(40.30)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>2.19</td>
<td>183</td>
<td>(46.10)</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.00</td>
<td>52</td>
<td>(13.10)</td>
</tr>
<tr>
<td>Trimethoprim-sulfonamides</td>
<td>1.23</td>
<td>324</td>
<td>(81.61)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>33.91</td>
<td>115</td>
<td>(28.97)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>47.11</td>
<td>225</td>
<td>(56.68)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>27.55</td>
<td>98</td>
<td>(24.69)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>34.97</td>
<td>163</td>
<td>(41.06)</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>36.18</td>
<td>152</td>
<td>(38.29)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1.33</td>
<td>376</td>
<td>(97.71)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>13.95</td>
<td>43</td>
<td>(10.83)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>12.50</td>
<td>144</td>
<td>(36.27)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>16.91</td>
<td>136</td>
<td>(34.26)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>9.57</td>
<td>94</td>
<td>(23.68)</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>7.42</td>
<td>283</td>
<td>(71.28)</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>1.77</td>
<td>113</td>
<td>(28.46)</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>0.00</td>
<td>39</td>
<td>(9.82)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>2.10</td>
<td>238</td>
<td>(59.95)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.00</td>
<td>231</td>
<td>(58.19)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.00</td>
<td>379</td>
<td>(95.47)</td>
</tr>
<tr>
<td>Quinupristin-Dalfopristin</td>
<td>2.13</td>
<td>94</td>
<td>(23.68)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.00</td>
<td>225</td>
<td>(56.68)</td>
</tr>
</tbody>
</table>

| Antibacterial susceptibility of Gram-positives | S463 |

**Conclusion:** *S. aureus* isolates from blood stream infections were highly susceptible for different compounds tested, whereas resistance was abundantly present (~25%) for β-lactams (including methicillin), fluoroquinolones, macrolides, and lincosamides. These data can guide empiric antimicrobial use in septicemic patients.
**P1631** Comparison of the in vitro activity of linezolid with erythromycin against coagulase-negative staphylococci isolates

A. Povita, C. Tuchilus, L.S. Iancu*, I. Badicuţ, D. Buică (Iasi, RO)

Linezolid belong to a new class of synthetic antimicrobial agents called the oxazolidinones. The spectrum of activity embraces Gram-positive organisms, including methicillin resistant staphylococci, vancomycin-resistant enterococci and penicillin-resistant pneumococci. The objective of the study was to determine the in vitro activity of linezolid against coagulase-negative staphylococci (CNS) isolates and compared it to that of erythromycin.

**Methods:** A total of 355 CNS strains were examined. All strains were collected from healthy individuals (nasal swabs and finger-clip platings) during 2007 year. The minimum inhibitory concentrations (MICs) for linezolid and erythromycin were performed by agar dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations. Resistance rates are reported using the NCCLS breakpoints for the fully susceptible category; moderately susceptible isolates are classified as resistant. Also the MIC of oxacillin was determined.

**Results:** Linezolid has the best activity when compared with erythromycin. Of the 355 strains reported only 0.85% were resistant to linezolid. The MICs for linezolid were consistently low <1 mg/L for majority of strains (51.2%). The rate of erythromycin-sensitive strains resistant to linezolid was significantly lower. Also the associated-resistance trend is indicated by elevated MICs for erythromycin and oxacillin-resistant CNS strains. Approximately 55% of the oxacillin-resistant CNS were resistant to erythromycin. The MIC of erythromycin against CNS strains ranges from 0.06 mg/L to 8 mg/L and the MIC 50 were two-fold higher than breakpoint for susceptibility.

**Conclusion:** Linezolid has excellent activity against CNS strains and may be useful in the treatment of staphylococci infections. The absence of associated-resistance with linezolid was clearly evident. With regard to oxacillin-resistant CNS, linezolid resistance was absent among these strains. The percentage of erythromycin resistance was 15.2% while oxacillin-resistant strains of CNS tend to be resistant to erythromycin, majority of oxacillin-resistant strains are also susceptible to erythromycin.

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**P1632** Reversal of oxacillin resistance in MRSA with phenothiazines

M. Rahbar*, H. Mehragan, S. Hadji-Nezhad (Tehran, IR)

**Background and Objectives:** The antibiotic resistance is now common place throughout the globe. One of the most highly problematic antibiotic resistance is that caused by methicillin resistant *Staphylococcus aureus* (MRSA) The aim of this study was to evaluate psychotropic therapeutics, especially phenothiazines antimicrobial activity and reversal of antimicrobial activity.

**Methods:** This study investigated the anti-MRSA activity of phenothiazines presence in Iranian market and the the interaction of these compounds with oxacillin. The minimum inhibitory concentration (MIC) of each of phenothiazines and as well as oxacillin against MRSA isolates was determined by the agar dilution method as recommended CLSI. The possible synergy between test substrate and the antibiotic was also investigated by this method employing combination of phenothiazines at their 1/2 MIC50 and oxacillin in the range of concentration ≤1/2MIC. Degree of synergy is yet to be established by the checkerboard technique. *S. aureus* ATCC 33591 (Mec A Positive) and *S. aureus* ATCC 29213 (MecA Negative) were used as standard strains.

**Results:** All MRSA isolates were inhibited by test phenothiazines at MIC 50 ranging from 12-256 μg/ml with order of thioridazine > trifluperazine > chlorpromazine > trifluoprazine > promethazine. Chlorpromazine, thioridazine, promethazine and perphenazine decreasing oxacillin MIC50 from 384 μg/ml to 24 μg/ml showed a significant synergy with these antibiotics. Trifluperazine and thalidomide however had variable synergistic effect with oxacillin against 10 clinical MRSA isolates. The study is still under way to determine degree of synergy for drugs and antibiotics.

**Conclusion:** This study revealed the there is a significant synergism between oxacillin and phenothiazines against MRSA.
defined as R to \( \geq 2 \) of the following: penicillin (PEN), cefuroxime (CFX), azithromycin (AZI), trimethoprim-sulfamethoxazole (SXT), and tetracycline.

Results: PEN-R and MDR rates among SP varied by patient location (PL): PEN-R = 7% LTC and OP, 12% IP, and 21% ICU; MDR = 22% LTC, 25% OP, 30% IP, and 40% ICU. Levofloxacin (LVX) MIC90’s remained at 1 mg/L against SP, regardless of PL and R phenotypes with overall susceptibility (S) rates of \( \geq 98\% \) across PL. Similar trends were noted for CIP, though isolates were less S to CIP (91%) than LVX (99%) overall. MIC90’s of the \( \beta \)-lactams (PEN, CFX, ceftriaxone [CRO], and amoxicillin-clavulanate [AMC]) were 2–4 fold higher against IP and ICU populations relative to OP and LTC. For the \( \beta \)-lactams, AZI, and SXT S was lowest for isolates from the ICU and IP populations (PEN: ICU 61%, IP 68%, LTC 73%, OP 68%; CFX: ICU 67%, IP 82%, LTC 91%, OP 88%; AZI: ICU 61%, IP 68%, LTC 73%, OP 68%; SXT: ICU 69%, IP 73%, LTC 82%, OP 77%).

Conclusions: The prevalence of MDR and PEN-R isolates was highest in EU among the ICU and IP populations. A trend towards decreased S when moving from OP to IP and ICU populations was apparent for all evaluated agents excluding fluoroquinolones (FQ). Regardless of PL, SP isolates overall remained \( \geq 97\% \) S to LVX and LVX remained the most potent agent tested against SP. Variation in S to \( \beta \)-lactams and macrolides and the consistent activity of FQ against SP from the OP to IP and ICU settings are factors which should be strongly considered when selecting an empiric agent for the treatment of respiratory infections.

### P1636

**Report of linezolid resistance from the Zyvox® Annual Appraisal of Potency and Spectrum Program (Europe, Latin America, Asia Pacific)**

J. Ross*, P. Hogan, D. Sheehan, R. Jones (North Liberty, New York, US)

**Objectives:** To monitor Linezolid (LZD)-resistance (R) strains, the Annual Appraisal of Potency and Spectrum (ZAAPS) Program (year 6; 2007) was initiated in various geographic areas of the world. LZD, the first oxazolidinone agent clinically applied, has become an important therapeutic addition for infections caused by antimicrobial-R Gram-positive (GP) pathogens. LZD-R has been observed particularly in enterococci (ENT) and recently among coagulase-negative staphylococci (CoNS), but occurrence rates are extremely low for indicated *S. aureus* (SA) and streptococci.

**Methods:** 5,591 GP strains were collected from 64 sites in 23 countries in 2007. Strains were received from the following organism groups: SA (3000), CoNS (716), ENT (906), *S. pneumoniae* (SPN; 452), viridans group (VGS; 155) and \( \beta \)-haemolytic streptococci (BHS; 362). At least 200 isolates from each country (expect China [800] and United Kingdom [400]) were requested to be sent to a reference laboratory for CLSI broth microdilution susceptibility (S) testing.

**Results:** The ZAAPS reports from 2006 and 2007 cited LZD-R *S. epidermidis* from the same hospital in Rome (Table).

Table: Linezolid-R isolates found in the 2007 ZAAPS Program

<table>
<thead>
<tr>
<th>Species</th>
<th>City/Country</th>
<th>LZD MIC (mg/L)</th>
<th>R-mechanism (23S mutation)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>Dublin/Ireland</td>
<td>8</td>
<td>G2576T</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>Rome/Italy</td>
<td>8</td>
<td>G2576T</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>Rome/Italy</td>
<td>8</td>
<td>G2576T</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>Brasilia/Brazil</td>
<td>&gt;8</td>
<td>G2576T</td>
</tr>
</tbody>
</table>

*Clonal.*

Further examination of other *S. epidermidis* isolates from the same hospital showed five other strains with LZD MIC values of 4 or 8 mg/L. MRSA rates ranged from 1.7% (Sweden) to 68.0% (Japan). Among vancomycin-resistant enterococci, Korea had the highest rate of 38.6%, SPN had overall penicillin and erythromycin resistance rates of 20.1% and 33.6%, respectively. All streptococci had LZD MIC values of \( \leq 2 \) mg/L. Overall LZD-R was 0.07% (0.12% in 2006). LZD-R rates among organism groups were: SA (0.03%), CoNS (0.28%), and ENT (0.11%).

**Conclusions:** LZD remained highly active against contemporary pathogens from indicated organism groups with an overall S rate of 99.83%. As LZD use continues to evolve, the need for close monitoring of the LZD in vitro activity versus Gram-positive pathogens and for the emergence of R is apparent.

### P1637

**A current look at the in vitro activity of oritavancin and vancomycin against isolates of *S. aureus* from both Europe and the US**

D. Draghi*, C. Pillar, D. Sahn, G. Moeck, F. Arhin (Chantilly, US; St. Laurent, CA)

**Background:** Reports have emerged regarding an upward shift in vancomycin (VAN) MICs against *S. aureus* (SA) and the impact of elevated VAN MICs on clinical outcome. Oritavancin (ORI), a lipoglycopeptide, is currently under development for the treatment of Gram-positive infections. It is important to understand ORI activity as it relates to SA isolates with elevated VAN MICs (should these isolates become more prevalent). This study evaluates the trend in VAN MICs observed among recent surveillance (SURV) isolates (2005–2008) and the activity of ORI activity against SURV isolates with defined VAN MICs (\( \leq 0.25/0.5/1/2 \) mg/L).

**Conclusion:** Zabo was the most active of all tested quinolones against clinical isolates of S. pn including levofloxacin resistant.
Methods: SA clinical isolates collected in 2005–2008 (n = 8,186) from geographically diverse SURV studies in the US and Europe (EU) were centrally tested by broth microdilution (CLSI; MT-AT) against ORI and VAN in accordance with CLSI M100-S18.

Results: With the exception of an increasing trend in the amount of isolates with VAN MICs of 1 mg/L from 2005–2007 in the US, no notable MIC creep for VAN was apparent among SURV isolates, with a consistent mode, MIC50 and MIC90 of 1 mg/L. Interestingly, SURV isolates from EU and the US in 2008 had more isolates with VAN MICs of 0.5 mg/L than in prior years. Similarly, a lower overall ORI MIC50/MIC90 (mg/L) in 2007 and 2008 was observed (0.03/0.06 US; 0.03/0.12 EU) relative to 2005 and 2006 (0.06/0.25 EU). Against SA with VAN MICs of 0.5 and 1 mg/L, ORI maintained potent activity with MIC50s from 0.03–0.06 mg/L and MIC90s 0.06–0.25 mg/L among evaluated SURV isolates from 2005–2008. However, ORI MIC50s and MIC90s against SA with VAN MICs of 2 mg/L (MIC50s of 0.12–0.25 mg/L; MIC90s 0.25 mg/L) were slightly higher relative to isolates with VAN MICs <1 mg/L over this period.

Conclusions: Apart from a slight upward shift in VAN MICs apparent with US isolates from 2005–2007, little evidence for VAN MIC creep was evident with isolates from large scale SURV studies. In fact, VAN MICs in 2008 tended to be slightly lower than those from 2005–2007. Regardless, ORI MIC50s and MIC90s were consistent and lower than VAN against SA with defined VAN MICs of 0.5, 1, or 2 mg/L, though ORI MICs were slightly elevated against the small subpopulation of isolates with VAN MICs of 2 mg/L. Future SURV is warranted to monitor for shift in VAN MICs and its effect, if any, on the activity of ORI.

**[P163]** Update on daptomycin activity and spectrum when tested against Gram-positive strains collected in European medical centres (2007–2008)

R. Jones, G. Moet, M. Castanheira, H. Sader* (North Liberty, US)

Objective: To evaluate the in vitro activity and spectrum of daptomycin (DAP) tested against recent clinical isolates collected in European hospitals. DAP is a cyclic lipopeptide approved by European Medicines Agency (EMEA) for the treatment of complicated skin and skin structure infections (cSSSI) and *S. aureus* (SA) endocarditis.

Methods: 10,430 consecutive strains were collected in 28 medical centres located in 11 European countries, Turkey and Israel. The following pathogens were evaluated: *SA* (27.4% oxacillin [OXA]-resistant [R]; coagulase-negative staphylococci [CoNS; 76.3% OXA-R], *E. faecalis* (EF; 1.2% vancomycin [VAN]-R), *E. faecium* (EFM; 25.6% VAN-R), *β*-haemolytic *Streptococcus* spp. (BHS; 807), and viridans group *Streptococcus* spp. (VGS; 274). The organisms were isolated from US excretions (74.2%) or one dilution above (25.8%). In contrast, VAN MICs in 2008 tended to be slightly lower than those from 2005–2007.

Results: DAP was highly active against SA and CoNS (MIC50/90, 0.25/0.5 mg/L for both organisms) and its activity was not adversely influenced by resistance to OXA (see Table).

Table:

<table>
<thead>
<tr>
<th>Organism (no. tested)</th>
<th>Cumulative % inhibited at daptomycin MIC (mg/L) of:</th>
<th>%S</th>
<th>%R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.12</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>SA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA-S (4,047)</td>
<td>0.5</td>
<td>86.5</td>
<td>99.7</td>
</tr>
<tr>
<td>OXA-R (1,531)</td>
<td>0.5</td>
<td>76.5</td>
<td>99.1</td>
</tr>
<tr>
<td>CoNS (1,665)</td>
<td>10.2</td>
<td>65.3</td>
<td>96.0</td>
</tr>
<tr>
<td>EF (1,306)</td>
<td>0.5</td>
<td>92.2</td>
<td>99.9</td>
</tr>
<tr>
<td>EFM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAN-S (533)</td>
<td>0.9</td>
<td>21.1</td>
<td>7.1</td>
</tr>
<tr>
<td>VAN-R (210)</td>
<td>0.0</td>
<td>1.4</td>
<td>4.3</td>
</tr>
<tr>
<td>BBS (897)</td>
<td>83.9</td>
<td>99.4</td>
<td>100.0</td>
</tr>
<tr>
<td>VGS (274)</td>
<td>31.4</td>
<td>65.7</td>
<td>92.3</td>
</tr>
</tbody>
</table>

MRSA varied from 1.4 in Sweden to 55.9% in Greece and showed high R rates to levofloxacin (87.7) and clindamycin (34.0%). DAP (MIC50/90, 0.25/0.5 mg/L) and VAN (MIC50/90, 1/1 mg/L) were active against all MRSA (100.0% S), and linezolid (99.9%) and TMP/SMX (98.6% S) was also very active against this pathogen. All EF were S to DAP (MIC50/90, 1/1 mg/L). VAN-R EFM was observed in 10 of 12 countries evaluated and was highest in Ireland (58.7%) and Greece (46.0%). Among VAN-R EFM isolates, only 80% were S to quinupristin/ dalfopristin and 43.3% showed high-level R to gentamicin. DAP was highly active against BHS (MIC90, 0.25 mg/L) as were most comparison agents tested. DAP was also very active against VGS (MIC90, 0.5 mg/L).

**Conclusions:** DAP showed significant potency and broad-spectrum activity against recent clinical isolates of Gram-positive organisms isolated in European medical centres, including R subsets. All organisms tested except for 2 CoNS were S to DAP and R to other compounds did not adversely influence the DAP potency against staphylococci, enterococci or streptococci.

**[P1639]** Potent antimicrobial activity of daptomycin tested against *Staphylococcus aureus* with vancomycin MIC of 2 mg/L isolated in United States and European hospitals (2006–2008)

H. Sader*, G. Moet, H. Becker, R. Jones (North Liberty, US)

Objective: To evaluate daptomycin activity (MIC and MBC) against *S. aureus* strains with elevated (2 mg/L) vancomycin (VAN) MIC values. Daptomycin is a cyclic lipopeptide approved by European Health Authorities for the treatment of complicated skin and soft tissue infections (cSSSI) and *S. aureus* bacteraemia, including right sided infective endocarditis.

Methods: A total of 410 *S. aureus* isolates with VAN MIC of 2 mg/L were collected from 50 United States (USA, 282) and European (EU) hospitals in the 2006–2008. MIC was assessed by reference CLSI broth microdilution method in cation-adjusted Mueller-Hinton broth supplemented to 50 mg/L of calcium for DAP tests.

Results: Daptomycin MIC distributions are presented in the Table. All EU strains were susceptible (S) to daptomycin and only 3.9% of strains from US exhibited elevated daptomycin MIC results (>1 mg/L). Overall, >90% of isolates had VAN MICs of <0.5 mg/L. Daptomycin exhibited potent bactericidal activity with MBC values at the MIC concentration (74.2%) or one dilution above (25.8%). In contrast, VAN MICs >32 mg/L in 12.9% of strains and 25.8% of strains tested for hVISA were positive.

Table:

<table>
<thead>
<tr>
<th>Region (no. of isolates)</th>
<th>Cumulative % inhibited at daptomycin MIC (mg/L) of:</th>
<th>MBC50</th>
<th>MBC90</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.25</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Europe (128)</td>
<td>31.3</td>
<td>91.0</td>
<td>100.0</td>
</tr>
<tr>
<td>United States (282)</td>
<td>29.4</td>
<td>91.4</td>
<td>96.1</td>
</tr>
</tbody>
</table>

**Conclusions:** *S. aureus* strains with VAN MIC of 2 mg/L showed high rates of hVISA and VAN tolerance. The vast majority of *S. aureus* with VAN MIC of 2 mg/L were susceptible to Daptomycin. Daptomycin also retained potent bactericidal activity against *S. aureus* with VAN MIC of 2 mg/ml.
Susceptibility trends for methicillin-resistant Staphylococcus aureus and methicillin-susceptible Staphylococcus aureus bloodstream isolates over a 2.5 year period

M.B. Perri*, C. Moore, S. Donabedian, N. Haque, A. Nacqi, M. Zervos (Detroit, US)

Objectives: To access changes in vancomycin (V) and daptomycin (D) minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible Staphylococcus aureus (MSSA) bloodstream isolates, including USA300 strains over a 2.5 year period.

Methods: We evaluated 360 MRSA (170 USA100, 146 USA300, 44 other) and 97 MSSA consecutive bloodstream isolates from patients in urban Detroit from January 2006 to June 2008 to determine susceptibility trends using susceptibility methods for MIC by manual microbroth dilution and Etest (bioMérieux, Inc.) to V, D and susceptibility to linezolid (L) by Etest. MBC was determined for V and D. Tolerance for V and D was determined by a >16 fold difference of MBC/MIC. Pulsed-Field Gel Electrophoresis (PFGE) was performed on the MRSA to determine the USA strain type as described by CDC. Trends through 10 quarters were analyzed for each of the methods. The geometric mean was determined for each quarter and analyzed over time.

Results: Statistical analysis was performed using Spearman’s correlation, and significant statistical increases or decreases are described in table 1.

For MRSA, and specifically both USA100 and USA300 strains, a significant decrease across time was detected in MIC of D, V and L using Etests, as well as MBC’ and tolerance for V. A significant decrease with the MSSA was seen in the D Etest, while a significant increase was noted across the time in the MIC using manual microbroth and MBC for V. Over the 2.5 year study period, 23% of MRSA and 13% of MSSA were tolerant to V, while 0% of the strains exhibited tolerance to D. There were no significant changes in MIC’s of MSSA to V (range 0.25–2) or D (range 0.12–2) over the study period by manual microbroth.

Conclusion: Over a 2.5 year period in urban Detroit, we found a significant decrease in MIC’s, MBC’s and in vitro tolerance of MRSA bloodstream isolates to vancomycin, linezolid and daptomycin, however, a significant increase was seen in the MIC’s and MBC’s to MSSA.

Antibiotic susceptibility patterns of Staphylococcus aureus in north-eastern Romania and comparison of phenotypic methods for detection of oxacillin resistance

O. Dorneanu*, V. Vremea, E. Miftode, E. Nastase, O. Filip, C. Dorobat (Iasi, RO)

Objectives: 1. To assess the antibiotic susceptibility patterns of methicillin-susceptible (MSSA) versus methicillin-resistant Staphylococcus aureus (MRSA) isolated from systemic or localised infections in North-East Romania. 2. To compare the accuracy of phenotypic methods for detection of oxacillin resistance in S. aureus.

Methods: We have tested by disk diffusion method (CLSI 2008) and with ATB Staph5 (bioMérieux, France) 668 S. aureus strains isolated from blood culture, cerebrospinal fluid, pus, sputum or urine from patients hospitalised in the Infectious Diseases Hospital, Iasi, Romania, between 1.01.2005–31.12.2008. Susceptibility to oxacillin was assessed by 5 methods: oxacillin disks, cefoxitin disks, ATB Staph5 (bioMérieux, France), oxacillin E-test strips (AB Biodisk, Sweden) and a penicillin-binding protein (PBP) 2a latex agglutination test (Slidex MRSA, bioMérieux, France).

Results: Oxacillin resistance rate was 46.9%. 17.6% strains showed oxacillin heteroresistance. More than one third (34.2%) of the tested strains had oxacillin minimum inhibitory concentration (MIC) over 256 mg/L and 37.1% had vancomycin MIC=2 mg/L. For oxacillin, MIC90 was over 256 mg/L and MIC 50 was 8 mg/L. For vancomycin, MIC90 was 2 mg/L and MIC50 1.5 mg/L. 17.1% of all strains present inducible resistance to clindamycin. MRSA strains showed multiple resistance to antibiotics (84.7% to erythromycin, 58.8% to tetracycline, 55.3% resistance to gentamycin, 36.4% to fluoroquinolones, but only 8.2% to ciprofloxacin). MSSA have low resistance rates to other antibiotics. All strains were susceptible to daptomycin, quinupristin-dalfopristin and linezolid.

Slidex MRSA, disk diffusion testing with cefoxitin disks and ATB Staph5 correlated very well with E-test results, considered gold standard (efficacy 100%, 99.4%, and 98.8%, respectively). Disk diffusion testing with oxacillin disks is a good, but less reliable method (efficacy 97%).

Conclusions: Resistance to antibiotics, especially to oxacillin represents a problem in the management of S. aureus infections. Vancomycin, linezolid and quinupristin-dalfopristin remain excellent alternatives for the therapy of severe infections produced by MRSA strains. Slidex MRSA, ATB Staph5 and cefoxitin disk diffusion are accurate methods for the assessment of oxacillin resistance.

In vitro activity of daptomycin against staphylococcal and streptococcal isolates from orthopaedic patients

S. Tsiplakou, V. Papaioannou*, A. Stylianakis, F. Zoumpaoueloglou, D. Paraskevopoulos, A. Koutoukou (Athens, GR)

Objectives: This study examines the activity of daptomycin and focuses on multi-trauma patients, who usually require prolonged hospitalisation and suffer mainly from complicated soft tissue infections. The aim of the present study is to determine the in vitro efficacy of daptomycin against staphylococci and streptococci, including multi-resistant strains isolated from patients with septicaemia, skin and soft tissue and implant-associated infections, and to provide a preliminary evaluation of the efficacy of the drug for infections in orthopedic patients.

Methods: All staphylococcal isolates were susceptible to vancomycin, linezolid and quinupristin-dalfopristin. Slidex MRSA, disk diffusion testing with cefoxitin disks and ATB Staph5 (bioMérieux, France) were used for quality control.

Results: All staphylococcal isolates were susceptible to daptomycin, linezolid, teicoplanin and vancomycin. The MRSA and MRSE isolates were multi-drug resistant, resistance phenotypes including resistance to β-lactams, erythromycin, clindamycin, gentamicin, fusidic acid, ciprofloxacin, minocycline, linezolid, teicoplanin, vancomycin, and sulfamethoxazole/trimethoprim were determined by the automated VITEK II system (Biomerieux, France), and MICs of daptomycin were determined by the E-test method (AB Biodisk, Solna, Sweden) according to CLSI guidelines. The S. aureus ATCC 29213 reference strain was used for quality control.

Conclusions: All staphylococcal isolates were susceptible to daptomycin, linezolid, teicoplanin and vancomycin. The MRSA and MRSE isolates were multi-drug resistant, resistance phenotypes including resistance to β-lactams, erythromycin, clindamycin, gentamicin, fusidic acid, ciprofloxacin, and sulfamethoxazole/trimethoprim. MIC values of MRSA and MRSE for daptomycin ranged from 0.19 to 1 mg/L, the majority of isolates showing MICs of 0.25 to 0.5 mg/L. MIC values of MSSA for daptomycin ranged from 0.19 to 0.5 mg/L, with the majority of isolates showing MICs of 0.125 to 0.25 mg/L. S. pyogenes isolates were susceptible to all antimicrobials tested, with the exception of...
sulfamethoxazole/trimethoprim, and the MICs for dapтомycin ranged from 0.047 to 0.5 mg/L, the majority of isolates showing MICs of 0.094 mg/L.

Conclusion: All Gram-positive clinical isolates tested, including multi-drug resistant isolates such as MRSA and MRSE, were susceptible to dapтомycin; which could be a new therapeutic option in the treatment of infections in orthopaedic patients.

Cleaning (ing) hospitals

P1643 Does targeted and quantified control of the microbiological environment within the intensive care unit reduce colonisation and healthcare-acquired infection?


Objective: There is currently little evidence to link inadequate cleaning with hospital-acquired infections. The aim of this study was to determine if a reduction in environmental MRSA contamination rates by enhanced cleaning led to a reduction in new colonisation of patients.

Methods: The study was conducted in the intensive care units of two London hospitals over one year and was divided into six eight-week phases randomised to ‘standard’ or ‘enhanced’ cleaning regimens. Enhanced cleaning was carried out by trained hygiene technicians who cleaned the high contact areas such as keyboards and bed rails in the near-patient environment twice a day using ultramicrofibre cloths. This cleaning regimen was in addition to the routine (standard) cleaning performed by the nurses. Conventional microbiological methods were used to sample 10 target sites three times a day. Samples were collected on 12 bed-days at each ICU each week. All patients were screened on admission and weekly during stay. Infections were defined using HELICS or CDC criteria.

Results: The proportion of patients who were MRSA-positive on admission to the ICU was similar at both Hospital A (124/1662; 7.5%) and Hospital B (88/921; 9.5%). However, the number of bed area samples from which MRSA was isolated was significantly higher in Hospital A (159/10052; 1.6%) than in Hospital B (76/10157; 0.8%, p < 0.001). Despite this, MRSA acquisition was 2.7 times more likely to occur at Hospital B (95% CI 1.6–4.6, p < 0.001). The total viable count recovered from target sites within both ICUs was significantly lower following enhanced cleaning than during the standard clean. The aggregated number of sites from which MRSA was isolated also fell significantly during the enhanced clean (165/10141; 1.6% vs. 70/10068; 0.7%, p < 0.001). However, in comparison to the standard clean, there was no evidence of reduced MRSA acquisition during enhanced cleaning at either Hospital A (10 vs. 12, OR 0.56 [0.10, 3.22]) or Hospital B (24 vs. 18, OR 0.72 [0.22, 2.38]).

Conclusions: Intensive cleaning of high contact areas within the near-patient environment reduced local bacterial load and potential risk to which the patient was exposed. However, within the limitations of this study, a lower level of environmental contamination was not associated with reduced colonisation or infection of patients.

P1644 Measuring the effect of enhanced cleaning in a UK hospital: a prospective cross-over study

S. Dancer, L. White, J. Lamb, E. Givran, C. Robertson (East Kilbride, Glasgow, UK)

Objectives: There is some evidence for cleaning in the control of hospital-acquired infections. This study aimed to evaluate the effect of one extra cleaner using microbiological standards based on aerobic colony counts and the presence of Staphylococcus aureus including meticillin-resistant S. aureus (MRSA).

Methods: We introduced one extra cleaner into two matched wards from Monday to Friday, with each ward receiving extra cleaning for six months in a cross-over design. Ten hand-touch sites on both wards were screened weekly using standardised methods and patients were monitored for MRSA infection throughout the year-long study. Patient and environmental MRSA isolates were characterised using molecular methods in order to investigate temporal and clonal relationships.

Results: Enhanced cleaning was associated with a 32.5% reduction in levels of microbial contamination at hand-touch sites when wards received enhanced cleaning (p < 0.0001: 95% CI 20.2%, 42.9%). There was little effect on environmental MRSA./S. aureus. Near-patient sites (lockers, overbed tables and beds) were more frequently contaminated with MRSA/S. aureus than sites further from the patient (p = 0.065). Genotyping identified indistinguishable strains from both hand-touch sites and patients. There was a 26.6% reduction in new MRSA infections on the wards receiving extra cleaning, despite higher MRSA patient-days and bed occupancy rates during enhanced cleaning periods (p = 0.032: 95% CI 7.7%, 92.3%). Adjusting for MRSA patient-days, and based upon nine new MRSA infections seen during routine cleaning, we expected thirteen new infections during enhanced cleaning periods rather than the four that actually occurred. Clusters of new MRSA infections were identified 2–4 weeks after the cleaner left both wards. Enhanced cleaning potentially saved the hospital up to £70,000.

Conclusion: Introducing one extra cleaner working Monday to Friday produced a measurable effect on the clinical environment, with apparent benefit to patients regarding MRSA infection. MRSA strains originally identified from hand-touch sites were later found in patients. There is scope for further research on hospital cleaning as a cost-effective component in the control of hospital-acquired infection.

P1645 Results of a 6-month in-use efficacy trial of AzoMaxActive™ cleaning products compared to hypochlorite


Objectives: The hospital environment acts as a reservoir for transmission of nosocomial pathogens, thus maintenance of a clean environment is essential for patient safety. Hypochlorite is recommended for cleaning areas at high risk for C. difficile as it is sporidical. However, it poses health and safety concerns and may damage the hospital environment. The AzoMaxActive™ range of cleaning products contain Brytrol™ technology, a novel combination of biocides sequestered to a nanopolymer backbone, which is sporidical in-vitro and displays residual antibacterial effect following use. The objective of the present study was to compare the antibacterial efficacy of AzoMaxActive to hypochlorite (Chlorclean), and to measure their effect on nosocomial infection.

Methods: The study took place in four identical acute medical wards each consisting of 5 four-bedded bays and 8 side rooms. Two control wards were cleaned with a regime of 1,000ppm hypochlorite on horizontal surfaces and neutral detergent on floors, two test wards were cleaned using the study products. Side rooms were not included in the study. Forty environmental sites per ward were sampled weekly. Two adjacent 25 cm² areas were swabbed per site. One swab was plated directly on blood agar for a total viable count (TVC), and then to Bower’s medium after enrichment in Robertson’s cooked meat medium to identify C. difficile. The second swab was inoculated onto chromogenic MRSA agar before and after enrichment in 7% salt broth. Numbers of patients identified as MRSA or C. difficile toxin (CDT) positive were monitored.

Results: A total of 2080 sites were sampled in each area, the number of sites with a TVC >10 or >100 or positive for MRSA or C. difficile are shown in the table. There was a highly significant difference in the proportion of sites with a TVC >10 or >100 in favour of the test areas. The difference was evident after 5 weeks and persisted thereafter. The number of MRSA or C. difficile positive sites was low and the differences not significant. Ten patients in the control area were C. difficile +ve and 8 in the test, for MRSA these figures were 7 and 9.

Conclusion: These 6 month results show that in use, the AzoMaxActive products have a significantly greater antibacterial activity than hypochlorite. The residual activity was also confirmed. Although the numbers are
small there appears to be no difference in *C. difficile* cases between the 2 arms of the study. The trial continues with a crossover phase.

<table>
<thead>
<tr>
<th>Sites +ve for</th>
<th>Test</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVC &gt; 10</td>
<td>1211 (58.2%)</td>
<td>1575 (75.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TVC &gt; 100</td>
<td>436 (21.0%)</td>
<td>627 (30.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MRSA Direct</td>
<td>31 (1.5%)</td>
<td>37 (1.8%)</td>
<td>ns</td>
</tr>
<tr>
<td>MRSA Total</td>
<td>45 (2.2%)</td>
<td>46 (2.2%)</td>
<td>ns</td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>12 (0.6%)</td>
<td>8 (0.4%)</td>
<td>ns</td>
</tr>
</tbody>
</table>

**P1646** High sporocidal activity using dissolved chlorine dioxide (SanDes™) on different surface materials contaminated by *Clostridium difficile* spores

J. Andersson, M. Sjöberg, L. Sjöberg, M. Unemo, T. Noren (Orebro, SE)

**Objectives:** To evaluate the sporocidal activity of dissolved chlorine dioxide (ClO2) on spore-concentrates from clinical *C. difficile* (CD) strains contaminating different surfaces in hospital environment.

**Methods:** Pure colonies of two CD strains; PCR ribotype 029 and 027/NAP1, both comprising high morbidity and sporulation capacity, were cultured anaerobically on Fastidious anaerobe agar (FAA). 8–10 colonies were then diluted in 1 mL NaCl, seeded into 30 mL prereduced peptone-yeast broth deficient of cystein, and cultured under anaerobe conditions imitating the in vivo nutritional starvation and transition to inactive spores. Maximum ratio of spores was seen after 5 days (60–80% of spores), according to calculations in Bürker-chamber using Phase-contrast microscopy.

On harvest the broth was diluted 1/100 in NaCl and 0.1 mL of this suspension was dried on glass, chrome metal, plastic and carpet. Subsequently, 0.1 mL 70% ethanol, 70% ethanol+200 ppm ClO2, 200 ppm, 400 ppm, 800 ppm or 1500 ppm ClO2, all in duplicates, were applied. The surface sample was then washed in 250 mL NaCl on a shaker for 20 minutes and filtrated through a 0.45 μm Millipore filter. Finally, the filter was cultured anaerobically for 48h on FAA and CFU count was recorded.

**Results:** The mean concentration of inoculum was 25×10⁹ cells/L (12–31×10⁹/L; 40–80% spores). The international epidemic strain 027/NAP1 showed the highest sporulation capacity (62–80%). In comparison with untreated contaminated surface sample, 70% ethanol gave a 30% mean reduction in CFU correlating with the proportion of susceptible vegetative cells, while this ethanol shock + 200 ppm ClO2 reduced growth by 97%. 800 ppm and 1500 ppm of ClO2 inhibited growth by 100%. Similar inhibition levels were seen for all surface materials examined.

**Conclusion:** This in vitro study demonstrated unique chemical disinfection of *C. difficile* spores, which may prove to be of great importance in hospital environmental cleaning from where infective CD spores are a major source of epidemic CD infection (CDI). Both clinical strains used, comprising high sporulating capacity, were effectively killed (97–100%) on the four surfaces tested when using ClO2 (200–1500 ppm) and may thus be used to clean floors, bedsrails (chrome-metal) or plastic WC-seats. Additional organic contamination and in vivo biofilm on surfaces need to be investigated as well as the value as hand-rub using ethanol 70%, which in this study did not show any sporocidal activity.

**P1647** The efficacy of dry-mist-generated hydrogen peroxide system against methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii*

N. Piskin, G. Celebi, C. Kulah, Z. Mengeloglu, M. Yumusak (Zonguldak, TR)

**Objectives:** The aim of this study was to evaluate the efficacy of dry-mist-generated hydrogen peroxide system (Sterinis®) against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* on the environmental surfaces in an intensive care unit room.

**Methods:** 0.5 McFarland suspensions of two test bacteria, either pure or containing 5% sterile serum were prepared. Inoculums of 5 μl from each suspension were inoculated on the sterile stainless steel disks separately. Each disk in a sterile Petri plate, either the plate cover closed or opened, was placed on the following locations: upper surface of the wardrobe, on the bedside table, into the drawer of whatnot and on the ground floor. Additionally, to test the efficacy of the Sterinis® on the later and underside surfaces of the equipments in the room, disks were placed in horizontal and prone positions using suitable apparatus. Duplicate disks for each location and for each position were placed. The system was run with a dose of 6 ml/mm² for one cycle according to manufacturer recommendations and quantitative cultures of each disk were performed after the cycle.

**Results:** Except one disk containing serum, no growth occurred on the disks with or without serum in the absence of a barrier, as a drawer or a covered plate or both, that limited air circulation. The presence of a barrier caused failure in the disinfection activity of the system: Cultures were positive for 8.3% of the disks in the opened plates and in 56.3% of the disks in closed plates. The difference was statistically significant (p < 0.001). Likewise, the rate of positive cultures inside the drawer was higher than the rate of positive cultures outside the drawer (4.2% and 68.8% respectively, p < 0.001). When the disks were compared according to serum content, disks with organic load had a higher rate of culture positivity but the difference was not statistically significant (Table 1). The results were similar when the analyses were performed for each bacteria separately.

**Conclusion:** Sterinis® was capable of killing MRSA and *A. baumannii* on the open surfaces in the hospital room with one cycle, however, presence of serum on the surfaces diminished the efficacy of it. Sterinis® was not effective in closed or semi-closed areas in the hospital room.

<table>
<thead>
<tr>
<th></th>
<th>Growth n⁺ (%)</th>
<th>No growth n⁺ (%)</th>
<th>Total n⁺ (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disks in</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>opened plates</td>
<td>4 (8.3)</td>
<td>44 (91.7)</td>
<td>48 (100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>closed plates</td>
<td>9 (56.3)</td>
<td>7 (43.8)</td>
<td>16 (100)</td>
<td></td>
</tr>
<tr>
<td>Disks outside the drawer</td>
<td>2 (4.2)</td>
<td>46 (95.8)</td>
<td>48 (100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>inside the drawer</td>
<td>11 (68.8)</td>
<td>5 (31.3)</td>
<td>16 (100)</td>
<td></td>
</tr>
<tr>
<td>Disks containing pure suspension</td>
<td>4 (12.5)</td>
<td>28 (87.5)</td>
<td>32 (100)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>serum + suspension</td>
<td>9 (28.1)</td>
<td>23 (71.9)</td>
<td>32 (100)</td>
<td></td>
</tr>
</tbody>
</table>

* n = The number of disks.

**P1648** Efficacy of selected weak organic acids against multi-antibiotic-resistant planktonic and biofilm bacteria

Z. Nack*, J. Stenos, H. Dunstan, S. Graves (Geelong, Newcastle, AU)

**Objective:** Determine the efficacy of selected weak organic acids (WOAs) and their combinations against planktonic and biofilm Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant Enterococcus faecium (VRE), Multiresistant *Acinetobacter baumannii* (MRAB) and extended-spectrum β-lactamase (ESBL) positive Klebsiella pneumoniae.

Four organic acids, ascorbic, citric, lactic and malic were tested alone or in various combinations.

**Methods:** For planktonic bacteria, an in-house test was developed. A time-concentration dependent study was carried out using individual and combinations of WOAs. For biofilm bacteria, bacterial suspensions were added to RPMI-1640 media supplemented with 1%v/v ethanol and
Efficacy of selected weak organic acids against enveloped viruses and an intracellular bacterium

Z. Nack*, J. Stemo, H. Dunstan, S. Graves (Geelong, Newcastle, AU)

Objective: Determine the efficacy of selected weak organic acids (WOAs) and their combinations against enveloped Herpes simplex type 1 (HSV-1) and non-enveloped Human adenovirus type 4 and against Rickettsia honei an intracellular, spotted fever group bacterium. Four organic acids, ascobic, citric, lactic and malic were tested alone or in various combinations.

Methods: Organic acids were diluted in RPMI-1640. We used different adherent cell lines, including Vero cells for HSV-1 and for Rickettsia honei and A549/88 cell line for Human adenovirus type 4 in 96 wells microtitre plates. The initial concentration of the viruses and the Rickettsia were determined by a ten-fold serial dilution method. Infected cell lines and cell lines exposed to low concentration of WOAs were used as negative control for all tests. Toxic effects of the WOAs on adherent cells were determined prior to experiment. Cell lines were grown up to form a monolayer and exposed to appropriate virus or bacteria for 48 hours. Individual and variation of WOAs in different concentrations were added to the cell lines for 60 minutes. After exposure, wells were washed with growth media (RPMI-1640) to remove any residuals of the WOAs and the same growth media was used to detect any viral or bacterial growth up to 14 days.

Results: The effective concentration of the WOAs to eliminate HSV-1 or Human adenovirus type 4 after 60 minutes exposure were as low as 0.16 w/v%. Our results showed no major difference in concentration to eliminate enveloped or non-enveloped viruses, although it may be due to the limited number of tests performed so far. Malic acid alone and in combination with citric and lactic acid was very effective against Rickettsia honei in 0.32 w/v% after 60 minutes exposure.

Conclusion: Our results indicate that WOAs alone or in combination work effectively against some enveloped and non-enveloped viruses and Rickettsia honei. They penetrate into adherent cells maintaining their efficacy without damaging the eukaryotic cells. The hazards associated with using these natural WOAs as sanitizer are probably significantly lower than the use of chemically based sanitizers because of their moderate or very low toxicity for humans.

For very fast and emergency sample analysis we developed one point calibration with high assay reliability.

Results: We successfully used this system for providing intensive care units within a distance of up to 200 km from our laboratory drug levels within 2–6 hours. Suggested dosage adjustments are delivered by us via appropriate PK-software. For samples from countries in Europe where a fast courier system is available, we were able to report drug levels within less than 24 hours of sampling. We have used this in 30 patients so far where the clinician considered drug concentration monitoring vital. In many of these cases the drug monitoring definitely optimised drug therapy leading to faster discharge. The effect on mortality cannot be assessed at this time but with more patients to enter we will be able to approach this most important question. Several cases will be presented in detail.

Conclusion: Modern bioanalytical technology is here, but its use in the everyday care of severely sick patients is still completely underdeveloped.

### P1652 Tigecycline by Lc-Ms/Ms in seven human intestinal tissues and comparison with rat and porcine tissue

S.T. Mees, V. Jakob, M. Rodamer, M. Kinzig, J. Haier, W. Haupt, V. Schellerer, F. Sörgel* (Münster, Nuremberg-Heroldsberg, Erlangen, DE)

**Objectives:** Due to the high volume of distribution of tigecycline in humans it is of great interest to study the distribution of tigecycline in various tissues in the body. Very few assays used for the measurement of antibiotics in tissue have been truly validated according the FDA-guideline for bioanalytical work. This was done here.

**Methods:** Determination of tigecycline in human colon (whole wall, mucosa), human duodenum (whole wall, mucosa), human ileum (whole wall, mucosa), human jejunum (whole wall), human stomach (whole wall, mucosa), human oesophagus (whole wall, mucosa), human liver, rat liver and porcine liver was performed with tandem mass spectrometric detection (LC-MS/MS) using TurboIonSpray® ionisation. Deuterated tigecycline was used as the internal standard. The following singly charged precursor → product ion transitions were monitored: 586 → 569 for tigecycline and 596 → 579 for d9-tigecycline. Sample preparation was performed by homogenisation and extraction with organic solvent. Chromatography was performed on a reversed-phase column with a mobile phase consisting of ammonium formate and ammonium acetate.

**Results:** Linearity could be shown within 0.00763–1.54 μg/mL and correlation coefficients were at least 0.998 on all validation days. Intra-run CVs ranged from 1.3 to 7.6% in human colon, 1.7 to 6.7% in human colon mucosa, 1.1 to 5.5% in human duodenum, 1.7 to 5.9% in human jejunum, 4.6 to 5.6% in human ileum, 1.3 to 6.7% in human liver, 1.4 to 8.2% in human stomach, 1.8 to 2.3% in human stomach mucosa, 1.2 to 11.2% in human oesophagus, 1.8 to 8.8% in human oesophagus mucosa, 1.9 to 9.4% in human duodenum mucosa, 2.1 to 6.5% in human ileum mucosa.

**Conclusion:** The inter-run precision (CV) ranged from 4.8 to 7.2% for tigecycline. Mean recovery was 68.1%±7.8% SD for tigecycline and 68.1%±2.3% SD for d9-tigecycline. There was no instability observed for tigecycline during the validation procedure. Six different human colon tissue samples showed no effect on the determination of tigecycline. We developed and validated a method for the determination of tigecycline in human tissues with high batch acceptance rate and according to the demanding FDA-guideline.

### P1653 Tigecycline and polymorphonuclear leukocyte function

A. Naess*, H. Andreosa, S. Sornes (Bergen, NO)

**Objectives:** Tigecycline is an antibiotic which achieves high intracellular concentrations in polymorphonuclear leukocytes (PMNs). To evaluate the effects of tigecycline on the function of human PMNs, PMNs from fresh whole blood were incubated with tigecycline dilutions (0.1 to 100 mg/L).

**Methods:** Phagocytosis and oxidative burst induced by Staphylococcus aureus, as well as PMN Fcepsilon-receptor complement receptors, were measured by flow cytometry.

**Results:** Tigecycline had no effect on the phagocytosis or oxidative burst induced by S. aureus. However, incubation with tigecycline was associated with small but statistically significant decreases in the density of PMN complement receptors CD11b and CD35 (all concentrations) and Fcepsilon receptors CD16 (100 mg/L) and CD32 (10 and 100 mg/L), but not in the percentages of receptor-bearing cells, except for small reductions in the proportions of CD16 positive cells at 10 and 100 mg/L of tigecycline.

**Conclusion:** Tigecycline was thus associated with decreased density of human PMN complement and (at high concentrations) Fegamma receptors. Although statistically significant, these differences were small and did not influence PMN function as measured by phagocytosis and oxidative burst induced by S. aureus.

### P1654 Epithelial lining fluid concentrations of antibiotics – methodological artifacts?


**Objectives:** In the desperate search for a better predictor of pharmacodynamic and clinical response epithelial lining fluid (ELF) has become most popular. However, bioanalytical methodology for the antibiotics in ELF has remained obscure because of hidden “proprietary issues” of the assays. That is completely unacceptable, although accepted by many journals. It virtually does not allow to assess the validity of the published work on ELF and thus makes it worthless. Any bioanalytical work should follow the FDA-guideline. Urea – a crucial factor when calculating ELF – concentrations from bronchoalveolar lavage concentration (BAL) was always measured by the clinical routine method that neither was ever designed or validated for this work. Hence it is not surprising that ELF-data are controversial with no meaningful relationship to pharmacodynamic properties of the antibiotics.

**Methods:** We developed and validated (according to FDA-Guideline) hyphenated chromatography/mass spectrometry methods for antibiotics and urea that were uniformly applied to the compounds reported. Patients were from an intensive care unit except for ceftiazidim. That should allow a long awaited valid comparison between agents.

**Results:** ELF to plasma ratio: Trovafloxacin: 2.75±1.93, Cefidoren: 0.32±0.19, Meropenem: 1.51±0.72, Piperacillin: 3.54±2.19, Tazobactam: 5.60±5.38, Vancomycin: 0.92±0.85, Linezolid: 1.81±1.83.

**Conclusion:** This data helps to differentiate between agents and reincarnate an agent like vancomycin which, due to inappropriate bioanalytical technology, was considered poorly penetrating into ELF. Based on our data β-lactams show differences that reflect their chemically different properties. For the first time it was shown by a specific chemical method that an oral cephalosporin can penetrate into ELF.

### P1655 Modelling the auto-inhibition of clarithromycin metabolism during repeated oral administration


**Objectives:** Clarithromycin decreases CYP3A4 activity and thus gradually inhibits its own metabolism as well as that of co-administered...
drugs. This study aimed at understanding the time course of these changes.

**Methods:** Plasma concentration time profiles of clarithromycin and its active metabolite, 14(R)-hydroxy-clarithromycin, from 12 young healthy volunteers after oral administration of a clarithromycin suspension (500 mg bid for 7 doses) were modelled by population pharmacokinetic analysis in NONMEM.

**Results:** Non-linearity of clarithromycin metabolism was considered during model development and metabolite disposition kinetics was assumed to be linear. The absorption kinetics of clarithromycin was best described by a Weibull function model. Pharmacokinetics of clarithromycin and its 14(R)-hydroxyl metabolite were adequately described by a one-compartment model each for clarithromycin and its metabolite as well as an inhibition compartment that reflects auto-inhibition of clarithromycin metabolism. Up to 90% of the apparent total clarithromycin clearance (60 L/h) was susceptible to reversible auto-inhibition, depending on the concentration in the inhibition compartment. The proposed semi-mechanistic population pharmacokinetic model successfully described the auto-inhibition of clarithromycin metabolism and may be used to adjust doses of other drugs metabolised by CYP3A4 that are co-administered with clarithromycin.

**Conclusion:** Simulations showed that for the 500 mg bid standard dose no further increase of exposure occurs after approximately 48 h of treatment. For a 1000 mg bid dose reaching steady state is expected to take several days and to achieve a 3.6-fold higher clarithromycin exposure compared to 500 mg bid. This evaluation provides a rationale for a safer and more effective therapy with clarithromycin.

**P1658** Effects of azidothymidine on apoptotic cell death: role of nuclear factor kB

C. Matteucci, A. Monutolo, E. Balestrieri, S. Grelli*, B. Macchi, A. Mastino (Rome, Messina, IT)

**Objectives:** We have recently shown that azidothymidine (AZT) affects caspase activation in peripheral blood mononuclear cells in absence of remarkable apoptotic cell death (Pharmacol. Res., 2008, Epub). To investigate mechanisms underlying this apparent paradox we investigated the action of AZT on cell death in a monocytumour cell line. In particular, we have focused our attention on the relationship between nuclear factor kB (NF-kB) activation and apoptosis modulation by AZT.

**Materials and Methods:** U937 cells wt and U937 cells stable transfected with a dominant negative murine IkBa (mIkBa) or ac control vector (pcDNA3.1), were treated with AZT or vehicle and, in some experiments, with AZT in combination with different inhibitors of NF-kB. Apoptosis was evaluated by flow cytometry analysis. Gene expression was evaluated by a SuperArray.

**Results:** U937 cells response to AZT raised a peak of apoptosis only at 48 h after treatment. SuperArray results, at 18 h, showed the up-regulation of both pro- and anti-apoptotic genes and also of some genes associated to DNA repair and cell survival. U937-mIkBa cells were more susceptible than U937-pcDNA3 to AZT-induced apoptosis, thus inhibition of the NF-kB system seemed to render target cells more susceptible to AZT-induced cell death. This was further confirmed by the fact that treatment of U937 cells with AZT + a pharmacological IkBa inhibitor increased their susceptibility to apoptosis.

**Conclusions:** Our data suggest that some of the paradoxical effects of AZT on apoptosis could rely on various mechanisms driven by modulation of NF-kB dependent genes belonging both to the apoptotic and to the anti apoptotic downstream signalling. Combination of AZT + NF-kB inhibitor could be a new interesting pharmacological approach to simultaneously control infection and dysfunction of cell growth.

**P1659** Combination therapy for multidrug-resistant *Acinetobacter baumannii* in a neutropenic murine pneumonia model

Z. Yuan, K. Ledesma, R. Singh, J.G. Hou, R. Prince, V. Tan* (Chongqing, CN; Houston, US)

**Objective:** We have previously demonstrated the feasibility of using combination therapy against a multidrug-resistant (MDR) *A. baumannii* (AB) clinical isolate in an in-vitro infection model (Lim, AAC 08), but the in-vivo relevance of the observations is not well established. In this study, we examined the utility of combination therapy in a neutropenic murine pneumonia model.

**Methods:** Female Swiss-Webster mice (20–25 g) were rendered neutropenic by 2 doses of cyclophosphamide on days −4 and −1; transient nephrotoxicity was induced by uranyl nitrate on day −2. Anaesthetized animals were infected with approximately 10^5 CFU of MDR AB intra-tracheally under laryngoscopic guidance. Serum TNF-α and IL-6 were measured 24 h after infection. Pharmacokinetics of amikacin, cefepime and levofloxacin in infected animals were determined by a single dose study. Animals were treated with placebo and various 2-agent combinations intra-peritoneally, 2 hours after infection for 96 hours. Quantitative assessment of bacterial burden in animal lung tissues was performed at baseline, after 24 h of therapy, and upon death or at the end of experiment. Relative effectiveness of various agent combinations was predicted previously by a novel mathematical model using a 3-dimensional response surface.

**Results:** Both serum TNF-α and IL-6 were found to be significantly elevated in infected animals, compared to controls (p < 0.001). Animals were given combination humanised drug exposures (amikacin 20 mg/kg every 24 h, cefepime 180 mg/kg every 8 h, and levofloxacin 150 mg/kg every 24 h). All infected animals expired after 80 hours if untreated. Different agent combinations were not equally efficacious; cefepime + amikacin was found to have the most favourable mortality rate (40%), as predicted by the mathematical model. Tissue bacterial burden at 24 h was consistent with the mortality data.

**Conclusion:** Our in-vivo results validated the utility combination therapy for MDR AB. Optimal (mathematical model-guided) combination therapy may be useful for MDR infections and deserves further evaluation.

**P1657** Post-antibiotic effect of various antibiotics on *Legionella pneumophila* strains isolated from water systems

S. Birtek séz Tan*, Z. Zeybek (Istanbul, TR)

**Objectives:** The aim of our study is to examine the PAE of azithromycin, clarithromycin, ciprofloxacin and levofloxacin against *L. pneumophila* strains isolated from several water systems of different buildings in Istanbul.

**Methods:** Azithromycin, clarithromycin, ciprofloxacin and levofloxacin MICs were determined by microbroth dilution technique as described by CLSI. PAEs were determined by a standard viable method where bacteria in the logarithmic phase of growth were exposed for 1 hour to antibiotics and antibiotics were removed by centrifugation and repeated washing. The PAE was defined as PAE = T − C, where T is the time (in hours) required for the count in the test culture to increase 1 log_10 above the count observed immediately after centrifugation and C is the corresponding time for the controls. Experiments were performed in duplicate.

**Results:** The MICs were determined in a range of 0.0156–0.0312 μg/ml for azithromycin, 0.0078–0.0156 μg/ml for clarithromycin, 0.0156 μg/ml for ciprofloxacin and 0.0078–0.0156 μg/ml for levofloxacin. The PAEs of *L. pneumophila* strains exposed for 1 h to 1 × MIC and 4 × MIC of azithromycin, ciprofloxacin, clarithromycin and levofloxacin ranged from 0.70 to 2.75 h and from 2.95 to 4.5 h; from 1.45 to 5.75 h and from 1.7 to 7.20 h; from 1.85 to 3.55 h and from 2.95 to 4.8 h; from 1.25 to 2.65 and from 2.0 to 4.75 h, respectively.

**Conclusion:** All of the antibiotics have PAEs on *L. pneumophila* strains. When the concentration of antibiotics were increased, the duration of PAE was prolonged. The duration of PAE was prolonged related to the increasing concentrations. The findings of this study may have important information for the optimal timing of the doses during therapy with these antibiotics.
Testing the antibacterial and antifungal activity of the species *Pelargonium roseum* and *Pelargonium graveolens*

C. Gălea*, C. Cseko (Targu Mures, RO)

**Objectives:** The objective of the present study is to test the antibacterial and antifungal activity of essential oils yielded by *Pelargonium roseum* and *Pelargonium graveolens*.

**Methods:** In order to test their antibacterial and antifungal activity, we made use of the radial diffusimetric method, impregnating round filter disks made of paper with essential oil yielded by the above-mentioned plants. Previously sterilised 6 mm diameter round disks were used. On each we dripped 0.01 ml essential oil, using a sterile semi-automatic micropipette. The paper disks were put in Petri plates, onto simple agarose cultures, which were preserved at 37°C in thermostat during 24 h. Reading was done by measuring the diameters of inhibition of microbial development around the round disks.

The microorganisms used for testing the antimicrobial activity of essential oils were selected from the standardised microorganisms collection of Tg-Mures Public Health Centre. We used the following bacterial strains:

1. Gram-negative bacteria: *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*;
2. Gram-positive bacteria: *Staphylococcus aureus*, *Enterococcus faecalis*;
3. Fungi: *Candida albicans*.

The intensity of their antimicrobial effect was tested in comparison with some referential antibiotics, in form of microcapsules for antibiogram (ciprofloxacin, cotrimoxazole, gentamycin, amikacin, cephotaxim, cephaloridin).

**Results:** The results obtained are presented in the table.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Minimal inhibitory concentration (MIC) (mg/L)</th>
<th>Blood flow rate (CLHDF) (L/h)</th>
<th>Colistin (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.93 (2.01)</td>
<td>2.45 (1.26)</td>
<td>2.35</td>
</tr>
<tr>
<td>2</td>
<td>1.08 (0.40)</td>
<td>5.5 (1.10)</td>
<td>2.35</td>
</tr>
<tr>
<td>3</td>
<td>3.29 (0.62)</td>
<td>3.19 (0.62)</td>
<td>2.35</td>
</tr>
<tr>
<td>4</td>
<td>30 (13)</td>
<td>68 (8)</td>
<td>2.35</td>
</tr>
<tr>
<td>5</td>
<td>2430 (383.4)</td>
<td></td>
<td>2.35</td>
</tr>
</tbody>
</table>

**Conclusion:** Both CMS and colistin are significantly eliminated by CVVHDF in these patients, resulting in insufficient plasma concentrations of colistin. This raises questions of the necessity and magnitude of dosage adjustment in this population of patients.
concentrations equal to their mean serum levels. MER and COL were applied because these were prescribed by the attending physicians.

Results: MIC50 and MIC90 of LVF was 64 and 128 microg/ml respectively. A more than 3 log10 bacterial effect of LVF was shown by concentrations of 5, 10 and 25 microg/ml against five, five and five isolates respectively at all times of growth. MER was bactericidal in nil isolates and COL in two isolates. Synergy defined as any more than 2 log10 decrease of bacterial growth compared with the most active single agent was shown between 5 microg/ml of LVF and MER in four, four, five and six isolates at 2, 4, 6 and 24 hours of growth respectively; between 10 microg/ml of LVF and MER in five, five, five and eight isolates respectively; and between 25 microg/ml of LVF and MER five, five, six and eight isolates respectively. Respective synergy between 5 microg/ml of LVF and COL was found in four, four, four and two isolates; between 10 microg/ml of LVF and COL in four, four, four and three isolates; and between 25 microg/ml of LVF and COL in four, five, five and three isolates.

Conclusions: Despite the great MIC values, LVF showed considerable in vitro bactericidal effect against MDRA pathogens of VAP when applied at concentrations close to ELF. Single MER and COL were not that effective but they acted in synergy with LVF. These findings reinforce the application of LVF for VAP by MDRPA.

**New insights into the most commonly studied drug interaction with antibiotics: pharmacokinetic interaction between ciprofloxacin, gemifloxacin and probenecid at renal and non-renal sites**


**Objectives:** Probenecid interacts with transport processes of drugs at several sites in the body. Although frequently used in any new antibiotic development program, there has been very little sophisticated modeling to understand probenecid’s action. We modeled gemifloxacin in plasma and urine with and without probenecid simultaneously and compared it to the simultaneous model of ciprofloxacin (CIP), its metabolite CIP-M1, and probenecid. This allowed us to compare the extent, time course, and mechanism of the quinolone-probenecid interaction at the renal and non-renal sites in plasma and urine. Additionally, we studied the effect of probenecid on the formation of CIP-M1.

**Methods:** We ran two randomised, two-way crossover studies in healthy volunteers (CIP 6M/6F; gemifloxacin 9M/8F). Study 1: 200 mg CIP as 30 min iv infusion without and with 3 g probenecid divided in five oral doses. Study 2: 320 mg oral gemifloxacin without and with 4.5 g probenecid divided in eight oral doses. Drug analysis by LC-MS/MS and HPLC. We used non-compartmental analysis and modeled the full time course of gemifloxacin and probenecid as well as of CIP, CIP-M1 and probenecid in plasma and urine simultaneously with WinNonLin(R). We used ANOVA statistics.

**Results:** Data are ratio of geometric means [90% confidence intervals] (*p<0.01). Addition of probenecid reduced the renal clearance to 35% [29–41%] of baseline for CIP, to 34% [27–43%] for CIP-M1 (estimated by modeling) and to 49% [47–51%] for gemifloxacin. Probenecid reduced the non-renal clearance to 81% [74–88%] for gemifloxacin and to 92% [86–99%] (p<0.008) for CIP. Pharmacokinetic modeling showed a competitive inhibition of the renal tubular secretion of CIP by probenecid. The affinity for the renal transporter was 3.8 fold higher (median) for CIP than for probenecid and 7.2 fold higher for gemifloxacin than for probenecid. Our models indicated that probenecid inhibited the non-renal clearance of gemifloxacin, but did not affect the non-renal clearance of CIP or the formation of CIP-M1.

**Conclusion:** Simultaneous modeling of the full time course of gemifloxacin and probenecid as well as of CIP, CIP-M1 and probenecid as expected was superior to non-compartmental analysis in providing insight into the mechanisms of the interactions. Probenecid inhibited the renal secretion of gemifloxacin, CIP and CIP-M1 and slightly decreased the non-renal clearance of gemifloxacin.
event with higher drug concentrations (p < 0.001). Concentrations of 5 mg/L and 15 mg/L were associated with probabilities of toxicity of 26 and 63%, respectively. There was no ITZ concentration identified that corresponded to a marked increase in the probability of an adverse event, meaning the population could not be divided into discrete groups with different probabilities of toxicity.

**Conclusions:** Adverse events are common with ITZ therapy, and show a statistically significant relationship with mean ITZ concentrations. These analyses demonstrate that ITZ has a narrow therapeutic window and TDM may therefore aid in the optimisation of treatment regimens, in order to maximise the probability of success and minimise toxicity.

**P1665** 
Oral pharmacokinetics of isavuconazole in liver impairment due to cirrhosis

A.H. Schmitt-Hoffmann*, R. Boos, E. Peterfai, D. Edwards, J. Spickermann, M. Heep (Basel, CH; Balatonfüred, HU; Detroit, US)

**Background:** Isavuconazole is an extended-spectrum azole administered orally or intravenously as a water-soluble pro-drug (WSA). Isavuconazole is currently under investigation in phase 3 studies in patients with systemic candidiasis, aspergillosis or invasive fungal infection with rare moulds. Isavuconazole is slowly eliminated by CYP-mediated clearance. Therefore, we investigated the oral PK of isavuconazole in subjects with hepatic impairment due to alcohol consumption.

**Methods:** Healthy volunteers and subjects with mild and moderate liver impairment received a single oral dose of WSA equivalent to 100 mg isavuconazole. Subjects were enrolled in three groups (n = 8) matched for age, gender, body weight and BMI. Pharmacokinetic parameters were derived using WinNonlin 5.1. The Tuckey test was used to assess the statistical significance of differences in isavuconazole pharmacokinetics.

**Results:** Average Child-Pugh scores of 5.2 and 7.6 were measured in subjects with mild and moderate hepatic impairment, respectively. Subjects with liver impairment had significantly lower systemic clearance (CL/F) of isavuconazole, compared with healthy subjects, accompanied by almost a two-fold increase in the half-life and the AUC (p < 0.05). Cmax was slightly decreased in patients corresponding to a modest increase of V/F (p < 0.05). A similar effect was observed after intravenous administration (ICAAC 2008, Poster A-007). The impact of liver impairment was not statistically different to results after intravenous administration shown previously or the gender.

**Conclusion:** Administration of isavuconazole to patients with mild or moderate hepatic impairment will require a dose adjustment compared to normal patients. The dose adjustment will be the same irrespective of the route of administration and the gender.

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**S475**

**P1666** 
Skin concentrations and pharmacokinetics of posaconazole after oral administration

G. Krishna*, L. Ma, E. Beresford, A. Tacakko, M. Martinho, X. Yu, S. Komjathy (Kentworth, Lenexa, US)

**Objectives:** The primary aim of this study was to determine the concentration of posaconazole (POS) in 4-mm skin punch biopsy in human subjects given POS 400 mg twice daily (BID) orally (PO). Secondary objectives were to compare skin concentration and plasma levels of POS to the MIC90 of relevant pathogens.

**Methods:** This was a randomised, single-centre, open-label study of POS in healthy adults. Subjects received 400 mg POS BID for 8 days with a high-fat meal. Blood samples for plasma POS level determination were collected at prespecified times on Day 1 and Day 8. From each subject, two skin samples were obtained, one immediately before or after both the first and last doses of POS. A MIC90 value of 250 mg/mL, which encompasses the majority of common dermatophytes [1], was used to calculate the PK/PD parameters AUC (0–24 hr)/MIC90 and time-above-MIC90 in plasma and skin.

**Results:** A total of 30 adult subjects (18 to 60 yr) were dosed. On Days 1 and 8, POS attained peak plasma concentrations at a median Tmax of 8 and 5 hours, respectively. On Days 1 and 8, POS peak skin concentrations were attained at 12 and 3 hours; peak skin concentrations were obtained from a single composite profile. On Day 1, the AUC/MIC90 ratio was 29 and 14 in plasma and skin, respectively. On Day 8, these AUC/MIC90 values increased to 149 and 187 in plasma and skin, respectively. On Day 8, POS concentrations in skin and plasma were several-fold higher than the MIC90 for the entire dosing interval. POS dosed at 400 mg BID PO was safe and well tolerated among healthy subjects.

![Figure 1. Mean plasma ± 1 SD and skin concentration-time profiles of posaconazole on Day 8 after oral administration of posaconazole (400 mg BID) in healthy subjects.](image-url)

**Reference(s)**


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**P1667** 
Inhibition effects of four antifungal triazoles (itraconazole, fluconazole, voriconazole and posaconazole) on specific activities of CYP3A4 in human liver microsomes

S. Baloul, Y. Nicoix*, P. Engel, D. Levêque, R. Herbrecth, G. Ubeaud (Strasbourg, FR)

Trizoles are inhibitors of various cytochrome P450 (CYP) isoenzymes such as CYP3A4, CYP2C9 and CYP2C19. Their inhibitory potencies are vastly different and also differ for the various isoforms. Itraconazole inhibits CYP3A4 stronger than fluconazole and voriconazole, in human liver microsomes. Few data exists in regards to the comparative inhibitory effects of posaconazole on CYP3A4.

**Objective:** To compare in vitro the inhibition effects of 4 antifungal triazoles (itraconazole, fluconazole, voriconazole and posaconazole) on specific activities of CYP3A4 via Ki values under identical experimental conditions.

**Methods:** The inhibition effects of the four antifungal drugs were assessed on a recombinant CYP3A4-mediated midazolam oxidation activities in human liver microsomes. Ki values were estimated from Dixon plots using the appropriate enzyme inhibition model by nonlinear regression. These studies were performed by using midazolam, a specific
substrate of CYP3A4 in presence and in absence of these antifungal drugs after incubation of liver microsomes and regenerating system after dosage of midazolam. The inhibition model was evaluated with ketoconazole as a positive control of CYP3A4 inhibition. At 25 mM and 75 mM ketoconazole, midazolam metabolism from 5 mM midazolam was inhibited by 46% and 87% respectively.

Results: The Ki values (±SD) of itraconazole, fluconazole, voriconazole and posaconazole were 16±2 µM, 221±2 µM, 151±4 µM and 215±14 µM, respectively (Figure 1).

![Graph showing Ki values of antifungal triazoles against CYP3A4 activities.](image)

Figure 1. Ki values of antifungal triazoles against CYP3A4 activities. A higher Ki indicates a lower inhibition of CYP3A4.

The inhibitory effect on CYP3A4 is the strongest for itraconazole (itraconazole > voriconazole > fluconazole = posaconazole) (p value = 0.024). Itraconazole was also determined to be a competitive inhibitor whereas fluconazole, posaconazole and voriconazole seem to induce a non-competitive or mixed-type inhibition. Our in vitro results show that posaconazole is a weak CYP3A4 inhibitor comparable in intensity to fluconazole as opposed to itraconazole or voriconazole, which induce a much stronger inhibitory effect. When compared with other extended-spectrum triazoles, this suggests that posaconazole may have a decreased potential of drug-drug interactions via CYP3A4 inhibition.

P1669 Effect of protein binding in the activity of voriconazole and anidulafungin alone or combined against Aspergillus sp. using a time-kill methodology


Objectives: To study the effect of the presence of physiological concentrations of human albumin and 75% human serum by concentrations similar to the Cmax obtained in serum after steady-state doses of voriconazole (VOR) (400/200 mg) and anidulafungin (ANF) (200/100 mg) against two Aspergillus fumigatus (MIC VOR=1 and MEC ANF=0.015 and 0.12 mg/L) and two Aspergillus flavus (MIC VOR= 1 mg/L and MEC ANF= 0.03 and 0.12 mg/L) strains.

Methods: Killing curves were performed with a final inoculum of approximately 10⁵ spore/mL and a final VOR and ANF concentration of 2.08 and 8.6 mg/L, respectively (Cmax) in different media: a) RPMI broth (Cmax); b) RPMI broth with 75% human serum (Cmax-S), and c) RPMI broth with 3.75 g/dL human albumin (Cmax-Alb). In parallel, killing curves with VOR or ANF concentrations (0.87 and 0.086 mg/L, respectively) corresponding to free-drug (CmaxF) were performed in RPMI broth considering the implications of protein binding for VOR and ANF, respectively. Control growth curves were performed in all media tested without antibiotics. Cultures were incubated at 35°C. At each time point (6, 10, 24 and 48 h) the metabolic activity was assessed using an XTT assay. All experiments were performed in triplicate.

<table>
<thead>
<tr>
<th>Strain/MEC (mg/L)</th>
<th>Cmax</th>
<th>Cmax-S</th>
<th>Cmax-Alb</th>
<th>CmaxF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. fumigatus 5/0.015</td>
<td>42.44</td>
<td>50.55</td>
<td>45.57</td>
<td>100.00</td>
</tr>
<tr>
<td>A. fumigatus 8/0.12</td>
<td>40.00</td>
<td>35.00</td>
<td>70.49</td>
<td>100.00</td>
</tr>
<tr>
<td>A. flavus 9/0.12</td>
<td>100.00</td>
<td>64.91</td>
<td>100.00</td>
<td>98.28</td>
</tr>
<tr>
<td>A. flavus 10/0.03</td>
<td>87.06</td>
<td>59.8</td>
<td>77.32</td>
<td>92.78</td>
</tr>
</tbody>
</table>

Results: A rapid decrease in the cellular viability was observed for all strains in VOR and VOR plus ANF curves independently of the presence of human serum or albumin in the medium. In ANF curves, cellular viability (%) at 48 h was:

Conclusions: Synergism could not be demonstrated due to the high activity showed by VOR. Theoretical extrapolation of active drug from total drug by using the protein binding rate seems a non accurate method to study antifungal activity considering the implications of protein binding.

Public health

P1670 UK infectious disease research network

M. Head* (London, UK)

Objective: The Infectious Disease Research Network (IDRN) provides an innovative infrastructure that promotes multi-disciplinary collaboration, provides information on funding opportunities, organises training
**P1671 Biological risk in a pharmaceutical dump: Brucella abortus strain viable after 20 years of disposal**

R. Koncan*, S. Bianchi, A. Amendola, M. Canuti, G. Tridente, G. Cornaglia (Verona, Milan, IT)

**Objective:** To evaluate the viability of the Brucella abortus B19 vaccine strain, recovered 20 years after storage in sealed vials

**Methods:** Material of potential biological risk have remained buried for years in the soil of the 12,000 square metres wasteyard of a pharmaceutical research institute in Milan (Istituto Sieroterapico Milanese, ISM). During the ISM reclamation, huge quantities of live vaccine against B. abortus were discovered in hermetically sealed vials. The procedure of reclamation of the ISM waste pit was carried out under strict safety conditions, using individual and environmental protection. Two vaccine types of B. abortus (in liquid and in lyophilised form) have been unearthed during the process of reclamation and subsequently analysed. A review of documentation revealed that all these vaccines had been produced by the ISM prior to 1975. To evaluate the viability of the bacterial strains, vial contents were first inoculated into Brain Heart Infusion (Oxoid). After visible growth the colonies were subcultured onto Columbia and MacConkey agar plates (Oxoid). Brucella species were confirmed by PCR using specific primers based on the AMOS test for Brucella melitensis and B. abortus (ICM, Nov. 1994).

**Results:** Both the liquid and the lyophilised vaccines demonstrated growth on Brain Heart Infusion. After 24 hours of incubation in 5% CO2, non-pigmented non-haemolytic colonies were observed on Columbia agar plates. The lyophilised strain also grew in MacConkey agar on the third day after seeding. Confirmatory identification was carried out by molecular methods. Fragments obtained with the AMOS test were sequenced and the BLAST analysis performed revealed that both lyophilised and liquid samples contained B. abortus biovar 1, i.e. the Buck 19 vaccinal strain.

**Conclusion:** Our results reveal the existence of a biological risk associated with the uncontrolled burial of pharmaceutical industry waste, such as live vaccines. Humans may inadvertently be exposed to products derived from vaccine manufacture by means of unintentional inoculation or other routes of exposure (aerosol, oral, injection).

It is noteworthy that Brucella is classified a class-3 organism and is a potential agent for bioterrorism according to the WHO guidelines.

**P1672 Phenotypic and genetic investigation of virulence and antibiotic resistance hallmarks in Escherichia coli strains isolated from Black Sea water on Romanian coast**

E. Panus*, C. Bleotu, M.C. Chifiruc, N. Rosoiu, V. Lazar, M. Mitache (Constanta, Bucharest, RO)

**Purpose:** The aim of this study was to investigate by phenotypic and molecular techniques the antibiotic resistance profile and the virulence markers of 100 environmental Escherichia coli aquatic isolated marine water in Constanta, Romania.

**Material and Methods:** The antibiosusceptibility tests were performed by disk diffusion method (CLSI, 2008) and phenotypic screening of β-lactamases. The presence of different antibiotic resistance markers was correlated with the plasmidial pattern of the analyzed strains. Eleven (11) virulence factors were tested by using specific culture media. PCR was performed for the following antibiotic resistance and virulence genes: blaTEM-like, blaSHV-like, blaOXA-like, blaCTX-M-like.

**Results:** The tested strains exhibited high susceptibility to imipenem, ceftriaxone, cefotaxime and nitrofurantoin (99%), tobramycin (98%), cefazidime (97%), gentamicin and amikacin (96%), ciprofloxacin (93%), nalidixic acid and chloramphenicol (92%) sulphametoxazole (89%) and high resistance levels to ampicillin (94%). The tested strains exhibited 1 and 8 antibiotic resistance markers, the most frequent associations being: AMP+SXT (10%), AMP+C (9%), AMP+CIP (8%) and AMP+NA (7%). Moreover, the tested strains exhibited resistance to heavy metals, i.e. to Zn (72%), Mn (98%), Cu (98%), Co (95%), and Ni (99%), 43.6% strains exhibiting resistance to β-lactam antibiotics proved to be positive for the presence of β-lactamases when tested by nitrocephin rapid test. The synergy test was negative for all tested strains. 25% of the tested strains exhibited at least one plasmid with variable molecular weights. Concerning the virulence hallmarks harbouring by the sea water strains, 44% exhibited capacity of adherence to the cellular substrate (adherence indexes of 30% with localised, aggregative and diffuse patterns) and inert substrata (60%). Unexpectedly, an important number of strains (70.37%) also exhibited invasion ability of HeLa cells demonstrating the potential of these strains to colonise the animal and human tissues and to initiate an infectious process. The tested strains produced also soluble virulence factors, i.e. mucinase (100%), lysozyme-carboxylase (93%), aesculin hydrolysis (67%), β-haemolysins (6%). PCR analysis confirmed the presence of the resistance and virulence genes in the sea water E. coli genome, demonstrating its contribution to the maintenance of the environmental reservoir of pathogenicity.

**P1673 Microbiological quality of air conditioning systems in cars**

R.P. Vonberg*, P. Gastmeier, B. Kenneswey, H. Holdack-Janssen, D. Sohr, I.F. Chaberny (Hanover, Berlin, Braunschweig/Wolfenbuttel, DE)

**Objectives:** Because of better comfort for the passengers, air conditioning (AC) systems are a common feature in new built automobiles these days. However, there is some concern about a potential risk for the health for example for immune-compromised passengers that might be deriving from AC systems, as its impact on the number of particles and microorganisms in the air inside the vehicle remains yet unknown.

**Methods:** The study was performed between January 2004 and July 2006 (30 months) on the campus of the university of Wolfsburg and Hannover Medical School. The following study cars were used: Volkswagen (VW) Passat (station wagon, diesel, built 1998, 110,000 km), VW Polo FSI (small car, gasoline, built 2003, 10,000 km), Seat Alhambra (small van, gasoline, built 1997, 175,000 km). All cars were equipped with an automatic air conditioning system. Operation modes (maximum capacity) using fresh air from outside the car as well as circulating air from inside the car were examined. The total number of microorganisms and the number of mould spores were measured by impaction in a high flow air sampler. Particles of 0.5 to 5.0 μm diameter were counted by a laser particle counter device. Samples were taken within the vehicle
before starting the AC system an after 20 minutes of use. Air outlets other than the ones examined were taped during sampling. Outside air samples were also taken to determine the bio-burden in the fresh air.

**Results:** Overall 32 occasions of sampling were performed. The concentration of microorganisms outside the cars was always higher than it was inside the cars. After starting the air conditioning system the total number of microorganisms was reduced by 81.7%, the number of mould spores was reduced by 83.3%, and the number of particles was reduced by 87.8%. There were no significant differences neither between the types of cars nor between the types of operation mode of the air conditioning system (fresh air vs. circulating air; Figure 1).

**Conclusion:** The airborne concentration of all parameters (total number of pathogens, number of mould spores, and number of particles) that were looked for in this study improved during utilisation of the car's AC system. Thus, we believe that there is no increased risk of infection for immune-compromised persons when driving in cars with AC systems also. Most probably the risk for an allergic reaction will be reduced during use also.

**Figure 1.**

**Conclusion:** The surveillance of organic farming, e.g., cheese production is necessary and can be done economically and fast with the described techniques.

**P1675 Carriage state of *Neisseria meningitidis* isolated from healthy individuals in West Pomeranian region of Poland**

M. Zienkiewicz*, M. Richter, M. Kadlubowski, W. Hryniewicz, S. Giedrys-Kalemba (Szczecin, Warsaw, PL)

*N meningitidis* are responsible for community-acquired invasive infections (meningitis, sepsis) with a high degree of morbidity and mortality. The source of infection is usually asymptomatic carrier. Carriage state of meningococci in the nasopharynx is estimated at 5–30% in the population, including pathogenic strains which are 1.4–1.6%. Colonising and infecting serogroups differ in countries and regions.

The aim of this study was to determine meningococcal carriage state in West Pomeranian region of Poland, the serogroup, susceptibility pattern and genetic relatedness of isolates.

**Methods:** The representative group of 1354 healthy individuals of 1 month to 87 years was studied. Nasopharyngeal samples were collected with cotton-tipped swab and subsequently cultured on chocolate agar + PolYvitex VCAT3. Meningococci were identified by using API NH (bioMerieux) and NEISSERIATest (Pyla Lachema) and serogrouped with latex agglutination tests: BD Directigen Meningitis (Becton Dickinson), Pastorex Meningitis (BioRad) and PCR method.

Susceptibility patterns to antibiotics: penicillin, ceftriaxone, rifampicin and ciprofloxacin were determined using E-tests. Molecular characterization was performed by PFGE method with SpeI restriction enzyme (Becton Dickinson), Pastorex Meningitidis (BioRad) and PCR method. Susceptibility patterns to antibiotics: penicillin, ceftriaxone, rifampicin and ciprofloxacin were determined using E-tests. Molecular character-

**Results:** The carriage rate of *N. meningitidis* in the region was estimated at 3.03% (41 individuals). 56.6% of carriers came from collectiveness, 78% were men and 86.49% smokers. Serogroup B was identified in 13 isolates, C – 9, Y – 3, W135 – 1, 15 strains were non groupable. Serogrouped strains were mainly isolated from the group of age: 15–24 years (7) and >40 years (8). MIC ranges for all isolates were: penicillin 0.047–0.25 (3 isolates: group B, C, Y possess reduced susceptibility, MICs 0.094–0.25), ceftriaxone 0.002–0.004, rifampicin 0.006–0.094, ciprofloxacin 0.006–0.016 μg/mL. Isolates showed a high genetic diversity – 7 PFGE clusters consisted of 2 to 4 strains and 22 unique profiles have been described. Serogrouped isolates belonged to genetic types and unique profiles also.

**Conclusions:** There are 3% carriers of *N. meningitidis* in the population of West Pomeranian region. About 50% isolates belonged to group B and C. All pathogenic isolates are sensitive to ceftriaxone, rifampicin and ciprofloxacin, the most to penicillin. The high genetic diversity among isolates is observed. Supported by Grant N404 033 31/1927.

**P1676 How widespread are antibodies to *Acinetobacter baumannii* and *Huemophilus influenzae* lipopolysaccharides in a healthy population in Scotland?**

R.F.S. Morgan*, 1 R. Fenton (Edinburgh, UK)

**Objectives:** Nosocomial pneumonia is currently a major problem in intensive care units worldwide. Little is known about how widespread exposure is to two of the major bacterial causes of nosocomial
Extended-spectrum \(\beta\)-lactamase producing Enterobacteriaceae in the faecal flora of Portuguese nursing home residents

D. Gonçalves, H. Ferreira* (Porto, PT)

Objectives: Our previous work in community clinical isolates alerted us for the finding of extended-spectrum \(\beta\)-lactamase (ESBL) producers in particular niches, as nursing homes. In that way, the aim of our study was the detection and characterisation of ESBL producing Enterobacteriaceae in the faecal flora of nursing home residents, in Northern Portugal.

Methods: Faecal samples of nursing home residents from the North of Portugal were collected from January to July 2008. Samples were suspended in BHI. Isolates were selected in MacConkey agar with ceftazidime (2 mg/L), cefotaxima (2 mg/L), and aztreonam (2 mg/L). Lactose fermenters were randomly selected and susceptibility to antimicrobial agents was determined by agar diffusion methods according to the CLSI guidelines. Screening of ESBL producers was performed by the double disk synergy test and confirmed according to the CLSI. Identification of the selected strains was achieved by API 20E \(\beta\)-lactamases were characterised by isoelectric focussing. Conjugation assays were performed with Escherichia coli HB101.

Results: Of 184 faeces samples of residents in 6 nursing homes in the North of Portugal we screened 48 ESBL producing Enterobacteriaceae isolates: 39 Escherichia coli, 3 Klebsiella pneumoniae, 2 Citrobacter freundii, 2 Enterobacter cloacae, 1 Enterobacter aerogenes and 1 Proteus mirabilis, showing an ESBL of pl > 8 alone or in combination with \(\beta\)-lactamases of pls 5.4 and 7.4. ESBL gene was successfully transferred coding a \(\beta\)-lactamase of pl > 8 alone or in combination with the other \(\beta\)-lactamases.

Conclusion: Our results showed that nursing homes are particular niches of community, acting as reservoirs of ESBL producing Enterobacteriaceae. \(\beta\)-lactamase isoelectric points, alert for the hypothesis of one successful track of community dispersion of CTX-M-15, in different combination with other \(\beta\)-lactamases, as in the CTX-M-15 producing Portuguese ST5131 Escherichia coli epidemic clone, that needs further research. This reality poses questions in terms of hospital admission of patients originating from nursing homes, relating to prevention of hospital dissemination of ESBL producers, colonising those patients. Also the inverse, spreading to nursing homes and community in general, from colonised elderly patients discharged from hospital when return to the ancient care facility or even to family home, might create a cycle of dissemination of ESBL producing Enterobacteriaceae and ESBL coding genes.

P1678 The epidemiology of Staphylococcus aureus nasal and throat carriage in a large community-based population in northern Norway. The Tromsø Staph and Skin Study


Objective: Staphylococcus aureus nasal carriage is associated with increased risk of bacteraemia and skin/soft tissue infections as well as atopic dermatitis. Studies suggest that the tonsils also may be a significant reservoir for the microbe. Our aim was to study the epidemiology of S. aureus nasal and throat colonisation and carriage in a large community-based population in North Norway.

Methods: A cross-sectional study was done as part of the sixth Tromsø Study in 2007–2008. Random samples of adult birth cohorts were invited to a health survey including clinical examinations, blood samples, and questionnaires on socio-demographic factors, lifestyle, health, chronic diseases and symptoms. The participation rate was about 66%. Nasal and throat swab cultures were performed in 3,996 participants for the assessment of S. aureus colonisation. A repeated set of cultures were performed in 2,986 participants for the assessment of S. aureus carriage (1,707 women and 1,279 men). Mean age was 54.5 years (range=30–87 years). Median length of the time interval between cultures was 28 days. All specimens were cultured within 24 hours on chromID S. aureus agar. Two carriage patterns for each site were distinguished: non-or-intermittent and persistent carriage.

Results: The prevalence of persistent S. aureus nasal and throat carriage were 25.1% and 6.0% respectively, and the results were almost constant across quartiles of the time interval between cultures (cut-off values: 18, 28 and 40 days). Considering culture results from both sites, 11.9% were defined as consistently persistent carriers, a minor group was single throat carriers (9.8%), and the majority was single nasal carriers (78.3%). Male sex was related to higher risk of nasal carriage (odds ratio, OR=1.91; 95% confidence interval, 95%CI=1.61–2.27; adjusted for age). Age was negatively related to nasal carriage (OR=0.91 per 10 years; 95%CI=0.85–0.98; adjusted for sex). S. aureus throat carriage was strongly related to nasal carriage (OR=3.98 for nasal persistent carriage vs non-or-intermittent carriage; 95%CI=2.90–5.46; adjusted for sex and age).

Conclusion: The nasal vestibulum is the major niche for S. aureus. Age and sex are predictors for nasal S. aureus carriage. The role of other potential risk factors in S. aureus nasal and throat carriage is currently under investigation. This project brings prospects to studies of the host-microbe-environment triad in S. aureus carriage, infection, and skin disease.

P1679 e-Bug: evaluation of the e-Bug educational pack in England, France and the Czech Republic


Objectives: To measure the effectiveness of the e-Bug pack in improving children’s knowledge in 4 key areas – Introduction to Microbes, Transfer of Infection, Treatment of Infection and the Prevention of Infection, when used within the National Curriculum in England, France and the Czech Republic.

Methods: Teaching, using the e-Bug pack, was given by junior and senior school teachers.
A minimum sample of 151 students from both age groups completed each of the questionnaires in a range of schools. Quantitative questionnaires were completed by all students, at three different time points, to assess student baseline knowledge, knowledge change and knowledge retention. Qualitative data was obtained via teacher focus groups, teacher questionnaires and student questionnaires. All analyses will be performed in STATA version 10.

Results: Preliminary findings of the quantitative data shows a knowledge change (P < 0.001) in all subject areas in the UK. At the time of writing this abstract, data for France and the Czech Republic have not yet been analysed. Qualitative data demonstrated that students in both age groups preferred the Transfer of Infection sections of the pack as these were more interactive ‘hands on’ activities and group work activities were preferred by the majority of schools. The Antibiotic Use and Vaccines were the least liked by students as these followed a more class discussion/comprehension format. The STI activity was the only activity liked 100% by both students and teachers. Photocopying student sheets in the pack was disliked due to high costs, however teachers found the whiteboard presentations very useful. Teachers also felt that the provision of more electron microscope images on the web would make this section of the curriculum more realistic for the students. All teachers liked the inclusion of background information for themselves although some would have preferred more detail.

Conclusions: The e-Bug teaching pack was effective at improving knowledge about micro-organisms, hygiene and antibiotic use however a variety of changes based on both student and teacher feedback are required. The data from this evaluation will be used to modify the packs further, ready for translation into a variety of European languages in January 2009.

Virtual microbiology laboratory – teaching and learning experience in the modern era
M. Hui*, L.P.L. Low, L.W. Lam, C.Y. Chan (Shatin, HK)

Objectives: Acquisition of microbiological knowledge is both intellectual- and skills-oriented. The traditional ‘wet-lab’ is an important platform to achieve this goal. The heightened interest in microbiology and infectious diseases, and the administrative clustering of many institutions, has led to increased number of students from geographically diversified locations. As a result, it is increasingly difficult to organise laboratory classes with limited resources such as laboratory space and number of teaching staff. To address the situation, a web-based “Virtual Microbiology Laboratory” was developed and evaluated for its effectiveness.

Methods: Starting 2007/2008, a web-based Virtual Microbiology Laboratory was set up for nursing undergraduate microbiology studies. Students’ performance and perception of the course were analysed for three consecutive academic years (2006/07, 2008/09; class-size ranged from 174 to 190). These web materials comprised of a series of 58 web images, 19 videos, 9 online quizzes and an anonymous forum. Access to this web was voluntary, but usage was logged. Students’ learning outcomes were assessed by course evaluation questionnaire (23 items on a 6-point Likert scale: 1 = strongly disagree, 6 = strongly agree) and examination. The course evaluation was conducted at the end of the course, but prior to the examination. The examination was paper-based with long-answer-questions and a-type MCQ, which were marked by the same panel of teachers.

Results: An improved perception on understanding of concepts and interest in microbiology was observed with the launch of Virtual Microbiology Laboratory. An improvement in scoring on the MCQ was also observed (2008/09 vs 2006/07, p < 0.05). However, scoring with long-answer-questions had declined (p < 0.05).

Conclusion: The development of Virtual Microbiology Laboratory has stimulated interest among undergraduate students. Their knowledge, however, when assessed by traditional examination method had yielded divergent results. Ability to recall factual information, as tested by a-type MCQ, is enhanced. Ability to assimilate information, and to present them in a logical written manner (as tested by long-answer-question) had declined. While web-based e-learning is a useful shotgun approach to improve the breadth of information, it may not replace classroom or bench-side teaching where in-depth discussion are generated, hypothesis- and theorising-skills are developed and practised.

Carbapenem resistance in Enterobacteriaceae

Emergence of multidrug-resistant Klebsiella pneumoniae producing KPC-type carbapenemase, Italy
T. Giani, M.M. D’Andrea, P. Pecile, L. Borgianni, P. Nicolotti, F. Tonelli, A. Bartolini, G.M.Rossolini* (Siena, Florence, IT)

Background: KPC-type carbapenemases have recently undergone a consistent dissemination in various geographic areas (e. g. New York, Israel, Greece, Colombia), where they represent an important mechanism of acquired resistance to carbapenems and other β-lactams in Klebsiella pneumoniae and other Gram-negative pathogens. Here we report on the emergence of KPC-producing K. pneumoniae in Italy.

Methods: Susceptibility testing was performed by broth microdilution and Etest. Carbapenemase activity was determined by spectrophotometry. β-lactamase genes were investigated by PCR and sequencing. Outer membrane proteins (OMPs) were investigated by SDS-PAGE and sequencing of the corresponding genes.

Results: K. pneumoniae FIPP1 was isolated in October 2008 from a post-surgical intra-abdominal infection in an inpatient at Florence University Hospital. The isolate showed a multiresistant phenotype including carbapenems (imipenem, meropenem and ertapenem MICs, >32 mg/L) other β-lactams, fluoroquinolones, amikacin, tobramycin and trimethoprim-sulphamethoxazole. Susceptibility was retained only to gentamicin, colistin and tigecycline. The patient had been empirically treated with a carbapenem-based regimen. After isolation of FIPP1, therapy was switched to tigecycline with a favourable outcome. A crude extract of FIPP1 exhibited carbapenemase activity that was not inhibited by EDTA. Molecular analysis revealed carriage of KPC-3- and SHV-11-encoding genes. Analysis of the OMP profile revealed the absence of k36 porin, which was due to gene inactivation by insertion of IS5-like sequence. This likely contributed to the high-level carbapenem resistance of FIPP1. Epidemiological investigations are underway to trace possible epidemiological links with settings where KPC-producing Klebsiella are known to be endemic.

Discussion: Carbapenem resistance in Enterobacteriaceae is still very uncommon in Italy, where only metallo-β-lactamases have occasionally been reported as acquired carbapenemases in enterobacterial species. To our best knowledge this is the first report on KPC-producing K. pneumoniae in Italy, a finding of major concern due to the spreading propensity that similar strains exhibited in other settings.

Emergence of KPC(+) K. pneumoniae strains in Greece: evaluation of detection methods

Objectives: Carbapenem resistance, as a result of the production of Klebsiella pneumoniae carbapenemase (KPC)-type β-lactamases is emerging worldwide. The purpose of the present study was to detect KPC (+) strains of Klebsiella pneumoniae, in a tertiary Greek Hospital, during 2008.

Materials and Methods: A total of 50 multidrug resistant clinical isolates of Klebsiella pneumoniae strains with a resistance phenotype compatible with KPC carbapenemase production were included in the study. More than 50% of clinical specimens were collected from...
Intensive Care Units (ICUs) and were of respiratory system origin. Strain identification and antibiotic susceptibility testing were performed by the automated Vitek2 system (Biomerieux, France). All strains were further tested for the production of various β-lactamas, both phenotypically (ESBL, double disk test, VIM EDTA test, E-test imipenem, Hodge test, boronic acid/imipenem disc test) and by PCR for the detection of blaKPC gene. 

Results: In 38 (72%) of isolates, the EDTA test was positive, the Hodge and boronic acid/imipenem tests were negative and imipenem MIC was >32 μg/dl, a phenotype indicative of a VIM carbapenemase production. In the remaining 12 (24%) isolates, the EDTA test was negative; however, the Hodge and Boronic acid/imipenem tests were positive and imipenem MIC was 8–32 μg/dl, thus indicating production of KPC β-lactamase. Most KPC(+) strains were isolated after October 2008, both from ICU and non-ICU patients, with or without history of previous hospitalisation. In all cases, phenotypic identification of KPC-carbapenemase production was confirmed by molecular techniques. Further genotype investigation revealed that all KPC(+) isolates had the same rep-PCR patterns. 

Conclusions: This is the first report of isolation and identification of KPC(+) Klebsiella pneumoniae strains in our hospital. More than 50% of these strains were collected from ICU patients, although infection control measures should be taken to avoid further dissemination throughout the Hospital setting. The microbiology laboratory could contribute to this purpose, by including phenotypic investigation of all multidrug resistant enterobacteriaceae in routine practice. Boronic acid/imipenem disc test is safe, quick and easy to perform, in order to detect KPC(+) strains.

Objective: MBL-producing K. pneumoniae is a major public health problem in several Greek hospitals. Recently KPC-possessing K. pneumoniae isolates have been described. We describe the recent widespread of KPC producers among carbapenem non-susceptible K. pneumoniae (CNSKP) clinical isolates in our hospital.

Methods: During May-December 2008 all K. pneumoniae isolates exhibiting elevated imipenem and/or meropenem MICs (>1 mg/L) were screened with combined imipenem-EDTA disk test, cefuroxime test and boronic acid disk tests. MICs were determined by Vitek2 and Etest. All isolates expressing a probable KPC phenotype were tested by PCR and sequencing assays. Patient records were reviewed for demographic characteristics, co-morbidities and antibiotic exposure prior to KPC isolation. 

Results: A total of 83 patients harbouring CNSKP isolates were identified. In 31 of them (37%) the presence of KPC-2 was confirmed by phenotypic and molecular assays. Records of patients harbouring KPC-producing isolates (18 males/13 females; mean age 66.4y, range 17–88y) showed that 25 were infected and 6 were colonised. KPC producers caused bacteraemic episodes in 11 (35%) patients. The median time from admission to KPC isolation was 17 days. KPC producers were detected during or after ICU-hospitalisation in 20 patients (64.5%). Prior hospitalisation (in other hospitals or private health care system) or nursing home residency was noted for 9/11 non-ICU patients. Among co-morbidities, cardiovascular and renal diseases (45% and 29%, respectively) prevailed. Prior antibiotic exposure analysis revealed that the majority of patients had received β-lactams/β-lactamase inhibitors (71%) and quinolones (67%). Tigecycline and colistin were mostly used as treatment regimens. The crude mortality was 40%. All but one single patient KPC producers were susceptible to gentamicin. Notably, meropenem MICs were within CLSI susceptibility range in 15 cases. Colistin-resistant isolates were recovered from 8 patients (26%), 6 of them had no prior administration of colistin. Elevated tigecycline MICs (4 mg/L) were detected in 2 cases. Long exposure to tigecycline was noted in one of these patients. 

Conclusion: KPC-producing CNSKP poses a new threat. Guidelines regarding the successful detection of KPC producers, advice on antibiotic policy and strict infection control measures are urgently needed in order to restrain a new hyperrdemic situation.

Objectives: To determine microbiological and molecular presence of the carbapenemases from isolates carbapenem-resistant K. pneumoniae and P. aeruginosa. 

Methods: A total of thirty-six isolates resistant to carbapenems belonging to Bush group II, molecular class A. KPC enzymes confer resistance to all β-lactam agents including penicillins, monobactams, and cephalosporins, as well as carbapenems. KPC β-lactamases have great potential to spread due to their location on plasmids. While initially limited to the eastern United States, KPC enzymes have recently been reported in France (FR), Colombia (CO), China (CN) and Israel (IL). In this report, we describe the detection of KPC enzymes in Klebsiella pneumoniae (Kpn) isolates collected in the 2004 to 2008 Tigecycline Evaluation Surveillance Trial (T.E.S.T.) from IL, Puerto Rico (PR), CO and Greece (GR). 

Methods: Kpn from GR, IL, PR, S. Africa (ZA), S. Korea (KR), Taiwan (TW), Italy (IT), Argentina (AR), Brazil (BR) and CO with meropenem (MER) or imipenem (IMI) MIC values of >4, or etrapenem (ERT) MIC values of >2 were screened by PCR for the presence of blaKPC, the gene responsible for the KPC enzyme. MICs were determined using manual broth microdilution following CLSI guidelines. 

Conclusions: Monitoring the world-wide dissemination of KPC outside of the US via the T.E.S.T. global surveillance program revealed isolates in four countries, including the first cases reported from PR and GR. KPC+ isolates from PR were collected in 2006, while all other KPC+ isolates were from 2007–2008. Since therapy of infections caused by these multi-drug resistant organisms is difficult, it is essential to monitor their spread into new regions of the world.
meropenem, imipenem, and ertapenem susceptibilities were determined by disk diffusion method. For detection of carbapenemases we used the modified Hodge test (using disks imipenem and ertapenem) and for detection of metallo-carbapenemases was performed double-disk synergy tests (DDSTs) using an IPM disk and an EDTA + meropenem disk. The identification of genes encoding of carbapenemases KPC and metallo-carbapenemases was performed by PCR.

**Results:** We observed variability of resistant profiles to carbapenems. Resistance to the three carbapenems tested (23 isolates of K. pneumoniae and 1 isolate of P. aeruginosa), resistance to ertapenem and meropenem and intermediate susceptibility to imipenem (3 isolates of K. pneumoniae and 1 isolate of P. aeruginosa), resistance to ertapenem and meropenem and susceptibility to imipenem (1 isolate of K. pneumoniae), resistance to ertapenem, intermediate susceptibility to meropenem and susceptibility to imipenem (1 isolate of K. pneumoniae) and finally resistance to ertapenem and susceptibility to meropenem and imipenem (5 isolates of K. pneumoniae). All isolates were positive for the Hodge test and no difference was observed when using a disk of imipenem and ertapenem. We founded that all isolates were negative for metallo-β-lactamases by PCR and DDST and all isolates were positive for carbapenemase KPC by PCR. Sequencing from amplification products confirmed the presence from KPC-3 in the vast majority of isolates.

**Conclusions:** Despite the variability in resistance profiles, all isolates were resistant to carbapenems. Our results indicate spread of KPC in isolates in different cities from Colombia. This is the fist report of KPC-3 from Colombia.

**[P1687] Outbreak of KPC-2-producing Klebsiella pneumoniae in a university hospital, Ribeirão Preto City, Brazil**

L.N. Andrade, E.C. Clímaco, J.C. Ferreira, R. Martínez, A.L.C. Durini* (Ribeirão Preto, BR)

**Objectives:** The main objective of this study was to investigate the resistance mechanism of carbapenem-resistant K. pneumoniae outbreak in a university hospital in the Ribeirão Preto city – Brazil.

**Methods:** 43 carbapenem-resistant K. pneumoniae were isolated from non-repeated patients in the Intensive Care Unit of “Hospital das Clínicas de Ribeirão Preto (HCRP)”, from April of 2007 to June of 2008. Identification and antimicrobial susceptibility profile of the isolates were evaluated with Vitek® System. Genetic profiles of carbapenem-resistant K. pneumoniae were determined by Pulsed Field Gel Electrophoresis (PFGE). Minimum inhibitory concentration (MIC) of imipenem, meropenem and ertapenem were determined using ETest. Modified Hodge test was performed to detect carbapenemase production. PCR and sequencing were used to investigate several carbapenemases encoding genes (blaKPC, blaGES, blaSPM, blaIMP, blaVIM) and their genetic environment. Conjugation experiment was utilised to investigate carbapenem resistance transfer.

**Results:** The isolates demonstrated susceptibility only to polymyxin B and tigecycline. PFGE revealed the spread of a unique clonal type. Imipenem, meropenem and ertapenem MICs values were, respectively, 8 mg/L, 8 mg/L and 32 mg/L. Modified Hodge test was positive, indicating carbapenemase production. PCR amplification and sequencing identified blaKPC-2 gene, flanked by insertion sequence ISKpn7 and ISKpn6. This genetic environment suggests that blaKPC-2 gene may be mobilised by transposon Tns4401, as published previously. Conjugation experiment demonstrated that blaKPC-2 gene and carbapenem-resistant phenotype was transferred to recipient strain.

**Conclusion:** An outbreak of KPC-2-producing K. pneumoniae took place in the HCRP. The spread of blaKPC genes was related with a unique clonal type of K. pneumoniae, however, this gene was associated with mobile elements and can be transferred to other bacterial species. The KPC-producing K. pneumoniae can be considered as the “select of select” of multiresistant bacteria and it can become pandrug-resistant in little time.

**[P1688] Detection of KPC enzymes in Klebsiella pneumoniae isolates from NY/NJ sites in the TEST Program**


**Background:** Carbapenem resistance in Klebsiella pneumoniae (KPN) is mainly due to the presence of an acquired carbapenem-hydrolysing β-lactamase known as Klebsiella pneumoniae carbapenemase (KPC). The KPC enzyme confers resistance to all β-lactam agents including penicillins, monobactams, and cephalosporins, as well as carbapenems. Since the initial discovery in North Carolina in 2001, KPC enzymes have been reported in several outbreaks in the eastern United States, as well as sporadic cases in France, Colombia, China and Israel. We evaluated 173 ESBL+ KPN isolates from New York and New Jersey (NY/NJ) collected in the 2004 to 2008 Tigecycline Evaluation Surveillance Trial (TEST) for the presence of the KPC gene via PCR.

**Methods:** Imipenem (IMI), meropenem (MER) and ertapenem (ERT) MICs were determined for 173 ESBL+ KPN from 25 sites in New York and New Jersey. A subset of isolates was screened for the presence of blaKPC by PCR. All MIC testing was done by manual broth microdilution following CLSI protocols.

**Results:** 88 of 173 (47%) ESBL+ KPN had carbapenem MICs of ≥2. Of these, 85 (96.6%) were positive for the KPC gene. 15 isolates with carbapenem MICs of <1 were also screened and all were negative for KPC.

<table>
<thead>
<tr>
<th>MIC (µg/mL)</th>
<th>ERT</th>
<th>MER</th>
<th>IMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥32</td>
<td>na</td>
<td>31/31(100)</td>
<td>na</td>
</tr>
<tr>
<td>≥16</td>
<td>66/66(100)</td>
<td>31/31(100)</td>
<td>18/18(100)</td>
</tr>
<tr>
<td>8</td>
<td>12/13 (92.3)</td>
<td>9/9 (100)</td>
<td>53/54 (98.1)</td>
</tr>
<tr>
<td>4</td>
<td>6/8 (75)</td>
<td>11/12 (91.6)</td>
<td>12/13 (92.3)</td>
</tr>
</tbody>
</table>

**Conclusions:** The emergence and rapid spread of KPC is of concern, as therapeutic options against these multi-resistant organisms are limited. K. pneumoniae with blaKPC have been present in multiple sites in the NY/NJ area since at least 2004. The prevalence of KPC may be underreported due to the widespread use of commercial automated testing systems. The TEST study isolates were identified through manual microdilution testing, and may give a more accurate depiction of the prevalence of these organisms.

**[P1689] Prevalence of extended-spectrum β-lactamases among Enterobacteriaceae strains from the United States (USA; 2007) and correlation with KPC carbapenemases**

M. Castanheira*, A. Waters, P. Strabala, L. Woosley, R. Mendes, R. Jones (North Liberty, US)

**Objective:** To determine the types and prevalence of ESBL enzymes among carbapenem-non-susceptible (CARB-Ns) and carbapenem-susceptible (CARB-S) Enterobacteriaceae isolates with the correlation of ESBL-types with KPC production.

**Methods:** As part of the SENTRY Antimicrobial Surveillance Program, Enterobacteriaceae collected in USA medical centres (2007) with elevated cephalosporin MIC values (≥2 mg/L) by CLSI broth microdilution test were screened for the presence of ESBL-encoding genes. CTX-M, VEB, PER, OXA-2-like, OXA-10-like, TEM and SHV encoding genes were tested by PCR. Amplicons were sequenced on both strands. Carbapenemases genes (encoding KPC, IMP, VIM, NMC-A/IMI, SMEs and OXA-48) were screened in isolates showing MIC values at ≥2 mg/L for imipenem and/or meropenem. Statistical analyses were performed using Epi-Info 3.4.1.

**Results:** Among 2,844 Enterobacteriaceae, 287 strains (10% of the total) displayed elevated cephalosporins MIC values. Among these, 215 belonged to four species: E. coli, K. pneumoniae (KPN), K. oxytoca and P. mirabilis. The 72 remaining isolates were AmpC-producing
Carbapenem resistance in Enterobacteriaceae

**Species (dominantly E. cloacae [72%]).** Fifty-two isolates showed elevated carbapenem MIC values associated with high MIC values for cephalosporins and KPC-production was detected in 45 strains (86%). At least 18 types of β-lactamases were detected: CTX-M (4 variants; Table), SHV (9), TEM (2), OXA (2) and PER-like. Over 60% of the Carb-S isolates carried ESBL genes, while 48% of the Carb-NS strains carried these genes (P = 0.04). However, a more limited variety of β-lactamases was found among Carb-NS isolates when compared to Carb-S. TEM-1 and SHV-encoding genes were significantly more prevalent among KPC-producing isolates (77% for both; P < 0.01) than in Carb-S strains (39% TEM-1 and 43% SHV). blaSHV-11 and blaSHV-12 were detected in 17 (37%) and 12 (34%) of the KPC-producers, respectively. All 35 KPN KPC-producing isolates detected in New York state sites also harboured TEM-1 and SHV-encoding genes, while 9 of the 12 KPN Carb-S carried both of these genes.

**Conclusions:** ESBL production was observed with the same frequency among Carb-S and Carb-NS isolates. However, KPC-encoding genes seemed to be more prevalent among SHV-producing isolates also carrying blaTEM-1. Furthermore, these enzymes were found in the majority of Carb-S isolates from the same medical sites, suggesting that Carb-S strains carrying blaSHV and blaTEM have likely acquired KPC genes.

<table>
<thead>
<tr>
<th>Organism group (no.)</th>
<th>β-lactamases types (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-M</td>
<td>TEM</td>
</tr>
<tr>
<td>OXA</td>
<td>Other</td>
</tr>
<tr>
<td>Carbapenem-susceptible</td>
<td>34 22 1 1 19 10 20 26 20 10 4 1</td>
</tr>
<tr>
<td>Carbapenem-non-susceptible</td>
<td>1 0 4 1 18 12 3 1 4 45 2 2 2</td>
</tr>
<tr>
<td>KPC-producing isolates</td>
<td>1 0 4 1 17 11 2 1 4 40 1 1 48</td>
</tr>
</tbody>
</table>

1. Includes KPC-producing isolates
2. Enzyme variants with extended spectrum activity (ESBLs) were in parentheses.

**P1689 Carbapenem-resistant Enterobacter cloacae harbouring IMI-2 gene in Finland**

S. Koivela*, J. Antikainen, M. Vuara, J. Kirveskari (Helsinki, FI)

**Objectives:** Biochemical methods have been considered insufficient to identify the carbapenem resistance mechanisms. To supplement these gaps, a Real-Time multiplex PCR was developed for screening of clinically significant carbapenem resistance genes among Enterobacteriaceae, Acinetobacter sp., and Pseudomonas sp.

**Methods:** The Real-Time PCR assay design was divided in two multiplex reactions. The first multiplex was designed to detect VIM 1–22, IMP 1–24, OXA-48, KPC 1–7, GES 1–10, SPM, NMC-A, IMI 1–2, SME 1–3, GIM-1, and SIM-1 genes. The other multiplex was designed for OXA genes with carbapenemase properties only, including OXA-24, -25, -26, -40, -72, OXA-23, -27, OXA-50, OXA-51, -64–71, -75–78, -83–89, -92, -94, -95, OXA-55, OXA-58, OXA-60, and OXA-62. SYBR Green chemistry was chosen to allow numerous genes to be detected within single reaction. A melting curve analysis was used to preliminarily identify putative molecular mechanism of positive samples, which were confirmed with sequencing with reference primers. This assay was used to screen putative ESBL strains systematically. Moreover, the screening assay is part of routine diagnostics to screen putative Enterobacteriaceae species with reduced susceptibility (meropenem disk diameter < 23 mm), or reduced MIC (I/R) to either meropenem or imipenem (MIC > 256), but interestingly not, however, resistant to third generation cephalosporins, or trimetoprim/sulfa.

**Conclusion:** Large reservoir of carbapenem resistance genes in environmental species combined with increasing carbapenem use provoke the risk of emergence of rare or new carbapenemase genes, which may remain undetected even though broad combinations of biochemical and molecular techniques are implemented. Using the new high-throughput screening assay an E. cloacae harbouring IMI-2 gene was identified. To our knowledge, an E. cloacae with IMI-2 gene has previously been reported only once, in China. Furthermore, this is the first reported Finnish Enterobacteriaceae strain harbouring a carbapenemase gene as well.

**P1690 Molecular epidemiology of VIM-1-producing Klebsiella pneumoniae in Italy**


**Objectives:** metallo-β-lactamases (MBLs) are emerging resistance determinants in Enterobacteriaceae nosocomial pathogens worldwide, with an epidemiology of production following country specific patterns of occurrence. The aim of this study was to investigate the presence of MBL determinants among MDR Klebsiella pneumoniae isolates from three Italian hospitals.

**Methods:** 30 non replicate K. pneumoniae isolates showing reduced susceptibility to carbapenems were collected during 2007–2008 from three hospitals. Imipenem (IPM) MIC was determined by E-test and broth macrodilution method (CLSI 2008 guidelines). MBL and extended-spectrum β-lactamase (ESBL) production were screened by the IPM-EDTA disc synergy test and the double disc synergy test, respectively. The genes encoding the MBLs were characterised by PCR and sequencing analysis. All strains were probed after isoelectric focusing (IEF) for ESBLs genes and genotyped by PFGE using XbaI. The MBL and ESBL genes transferability was investigated by conjugation. Plasmids were characterised by RFLP analysis.

**Results:** 14/30 strains, characterised by IPM MICs ranging from 2 to 128 mg/L, resulted positive to the IPM-EDTA disc synergy test and PCR experiments detected blaVIM-1 like genes. In all strains blaVIM-1 gene was on a conjugative plasmid and blaSHV-5 and blaCTX-M-1 genes were cotransferred in 11 and 3 cases respectively. VIM-1 producers were isolated from the ICU of the S.Giovanni Rotondo hospital, from a Pavia Rehabilitation facility and from 6 different wards of the Pavia hospital. These latest strains clonally related by PFGE harboured different conjugative plasmids 80–90 kb large, on the contrary an apparent identical conjugative plasmid 85 kb large was characteristic of the clone detected in S. Giovanni Rotondo hospital. Two of the clones detected were responsible for outbreaks, but none for inter-hospitals diffusion. The PFGE fingerprint software analysis showed a notably difference between K. pneumoniae VIM producers previously detected in Bolzano and Genova Italian areas and the three clones of our study.

**Conclusions:**: This is the first report on the emergence of a MDR clone of K. pneumoniae producing VIM and CTX-M transferable enzymes. Control measures including screening by IPM-EDTA disc synergy tests should be applied to detect the MBL producers and to contrast the vertical and plasmidic diffusion of carbapenem-resistant K. pneumoniae in acute care and rehabilitation facilities.

**P1691 First blaVIM positive metallo-β-lactamase producing Enterobacter cloacae isolate in Germany**


**Objectives:** The frequency of metallo-β-lactamase (MBL) producing strains of Pseudomonas spp. is increasing worldwide. Nowadays, also MBL producing Enterobacteriaceae are detected in clinical specimens creating significant therapeutic problems, since these strains are not only
resistant to all β-lactams but often posses further resistance mechanism. Here we report about an Enterobacter cloacae strain with elevated MIC values against carbapenems isolated from clinical specimens at an University Hospital in Germany.

**Methods:** A Gram-negative rod isolated from multiple blood cultures of a haematological patient of the University Hospital of Leipzig, Germany was identified by the ID 32 E system. MICs against 23 antimicrobial agents were determined by broth microdilution. Furthermore, MBL test was carried out by Etest and PCRs were performed for blaVIM and blaIMP genes.

**Results:** The isolate was identified as E. cloacae. MICs for imipenem, meropenem and ertapenem were 4mg/L, 4mg/L, and 8mg/L, respectively. MIC for imipenem determined by Etest for MBL detection was >32mg/L and for imipenem plus EDTA 2mg/L. Thus, the strain was identified as a MBL producer. The PCR was positive for the blaVIM but negative for the blaIMP gene.

**Conclusion:** To our knowledge we report here the first blaVIM positive MBL producing E. cloacae isolated from clinical specimens in Germany.

**Reference:**


**Objectives:** The emergence of carbapenemases in Enterobacteriaceae is becoming a matter of concern. We studied the microbiological characteristics and epidemiological data of VIM-1 producing K. pneumoniae (VPKP) isolates recovered from clinical specimens of patients hospitalised in Belgian hospitals.

**Methods:** Antibiotic susceptibilities were determined using standard agar diffusion, VITEK2 and Etest MIC determination. The production of metallo-β-lactase (MBL) was examined by synergy testing with imipenem and EDTA. Isolates were typed by PFGE. Detection of blaVIM-1 and mapping of the VIM-1 encoding integrons were performed by PCR-sequencing. β-lactamase activities were analyzed by IEF and spectrophotometry.

**Results:** Between 06/08 and 08/08, 5 patients with VPKP isolates were identified in 3 teaching hospitals in Brussels. At hospital A and B, VPKP were detected from rectal swabs by screening upon admission in 2 patients transferred from a Greek hospital where they had been treated in an ICU. At hospital C, VPKP were found in clinical specimens (post-operative wounds [2], lower respiratory tract [1]) in 3 patients with severe underlying diseases hospitalised in 3 units. All patients were colonised and none of them developed an infection. No common epidemiological transmission link could be established between any patients. By PFGE, the 5 isolates clustered in 2 clones and 4 variants. All VPKP isolates showed a positive synergy test with EDTA, had high resistance level to meropenem and imipenem (MICs >32mg/L) detected by disk diffusion and automated systems. They were pan-resistant but susceptible to aztreonam and gentamicin only; 4/5 strains were also resistant to colistin (MIC> 16mg/L). Tigecycline was active against 4/5 strains (one isolate with intermediate resistance, MIC> 4mg/L). blaVIM-1 was part of a class 1 integron that also carried aacA7, dfrH1, aadA1 and sul1. After implementing additional contact precautions and a nationwide alert system, no new cases were observed after 4 months of surveillance in the concerned hospital. Only one additional sporadic case was detected at another hospital.

**Conclusions:** Emergence of VIM-1 MBL-producing, pan-resistant K. pneumoniae is reported for the first time in Belgium. This event confirms the potential risk of spread of multiresistant bacteria with international transfer of patients between acute care hospitals and highlights the value of early screening and control measures to contain their spread.
Results: In total, 12 carbapenem-resistant isolates, nine isolates of P. mirabilis and three isolates of K. pneumoniae, were recovered. All these isolates were MBL positive and expressed MICs for both imipenem and meropenem of $>$32 mg/L and simultaneously produced ESBLs. All P. mirabilis MBL positive strains were isolated from clinical samples of patients in one surgical ICU while 3 of MBL positive isolates of K. pneumoniae were isolated from clinical samples of patients at Clinic of Urology. Seven of nine MBL positive P. mirabilis strains belong to IPM family, and two other MBL positive strains belong to VIM family. Interestingly, three MBL positive strains of K. pneumoniae belong to SPM family of MBL. All patients from whom MBL positive Enterobacteriaceae were recovered had already had carbapenem therapy and central venous catheter.

Conclusion: For the first time we recovered MBL producing Enterobacteriaceae in our hospital. All these isolates expressed high MICs for both imipenem and meropenem and produced ESBLs. They belong to IMP, VIM and SPM family.

**P1697** Recurrent urinary tract infections due to metallo-$\beta$-lactamase-producing Klebsiella pneumoniae in the community setting

A. Poulopoulou*, F. Markou, G. Vrioni, S. Pournaras, P. Rozi, A. Tsakris
(Serres, Athens, Larissa, GR)

Objectives: Metallo-$\beta$-lactamases (MBLs) constitute a major public health problem limiting the therapeutic options. Until now these enzymes were considered to be confined in the hospital setting. We present the emergence of VIM-1 producing K. pneumoniae causing recurrent community-onset urinary tract infections (UTIs).

Materials and Methods: During July 2007-February 2008, carbapenem-resistant K. pneumoniae (CRKP) isolates were identified to cause UTIs in 12 outpatients (mean age 73 y). All had a history of previous hospitalisation due to genitourinary pathology or urologic surgical intervention. In 2 of them CRKP isolates were firstly isolated during their hospitalisation. Among the remaining outpatients, 8 had the first CRKP isolation within 30 days after hospital discharge and 2 within 3 months after hospital discharge. Identification and susceptibility testing was performed with Microscan System (Dade Behring) and E-test. Double disk synergy test using imipenem-EDTA and E-test MBL were used for phenotypic MBL detection. MBL genes were characterised by PCR and sequencing analysis. Medical records were reviewed to access patient's characteristics.

Results: Imipenem and meropenem MICs ranged from 8 to $>$32 mg/L. All isolates were susceptible to aminoglycosides, aztreonam and colistin but resistant to other $\beta$-lactams, trimethoprim and fluoroquinolones. One strain exhibited resistance to tigecycline. MBL phenotypic tests were positive for all 12 single patient CRKP isolates and molecular testing detected VIM-1 carbapenemase in all cases. Previous antibiotic exposure included quinolones and/or ticarcillin/clavulinate during hospitalisation and oral quinolones after hospital discharge. Seven of the outpatients were successfully treated with gentamicin or amikacin. The remaining 5 outpatients had recurrent UTIs due to VIM-1-producing CRKP for a period of 3 to 8 months. Medical records revealed that 3 of them had a history of bladder cancer and 2 of lithiasis.

Conclusions: The emergence of community-onset, MBL-positive K. pneumoniae infections among outpatients with a previous hospitalisation is an awesome issue. Predisposing factors, such as malignancy and lithiasis, were associated with failure of antimicrobial treatment and prolonged carriage of VIM-1-producing CRKP. Infection control measures and continuous surveillance are essential for the detection and successful treatment of infections caused by such multidrug-resistant pathogens in the community.

Objectives: Carbapenem resistance in Enterobacteriaceae can be mediated by a production of several carbapenemases or by a decreasing permeability of outer membrane of the cell wall combined with a production of some extended-spectrum (ESBL) or AmpC $\beta$-lactamase. The objective of this analysis is to characterise carbapenem resistant strains of Klebsiella pneumoniae in one of the biggest Czech hospitals, the University Hospital in Plzen.

Materials and Methods: All non-repetitive isolates of K. pneumoniae resistant to carbapenem were collected during the period of January 2007 and June 2008. In these isolates, ESBL, AmpC and MBL production were preliminary identified by DISP and carbapenemase production was verified by a spectrophotometric assay. The isolates were typed by PFGE and MLST. ESBLs and AmpCs were characterised by isoelectric focusing, bioassay, and PCR and sequencing of bla genes. Plasmids were typed by PBRT. Porin analysis was done by a selective isolation of these proteins followed by SDS-PAGE and western-blotting with specific antibodies against OmpK35 and OmpK36, OmpK35, OmpK36, and OmpK37 genes were amplified and sequenced.

Results: During described period, six resistant strains were found. Isolates with the same $\beta$-lactamase content, but susceptible to

**P1696** OXA-48 carbapenemase-producing Klebsiella pneumoniae in the UK

J. Zhang* (London, UK)

Objectives: OXA-48 carbapenemase has been found mainly in K. pneumoniae and other Enterobacteriaceae in Turkey, rarely from elsewhere. We characterised the first 10 isolates of K. pneumoniae with OXA-48 enzyme referred from UK hospitals.

Methods: Bacteria were identified by API20E. MICs were determined by agar dilution according to EUCAST/BSAC protocols. Isolates were compared by PFGE of XbaI genomic digests. $\beta$-lactamase genes and IS elements were sought by PCR with sequencing of selected amplicons. Plasmids were extracted by alkaline lysis.

Results: Ten isolates of K. pneumoniae with OXA-48 enzyme were received from 6 patients hospitalised in four UK centres as follows: Patients in centre 1, 5 isolates from a patient transferred from Turkey; centre 2, 4 isolates from 3 patients; Centre 3, 3 isolates from 2 patients; Centre 4, 1 isolate from a patient transferred from centre 2. Patients in centres 2, 3 and 4 had no links with Turkey. Isolates were from wounds (6), urine (3) and blood (1). All patients had PFGE-distinct strains except in centre 3 where there was evidence of cross-infection. All isolates were resistant to amikacin and tobramycin (MIC $>$16 mg/L) and non-susceptible to imipenem (MICs $>$128 mg/L), but 3 remained susceptible to meropenem (MICs 1–2 mg/L). A blaOXA-48 gene was detected in all isolates, flanked on both sides by IS1999. Nine isolates had blaTEM and blaSHV genes, 4 had both blaOXA-1 and blaCTX-M group 1 genes; none had blaOXA-9. The 4 isolates with blaCTX-M were resistant to tobramycin and amikacin, but susceptible to gentamicin, consistent with AAC(6')-I activity, and were resistant to ciprofloxacin; 5 of 6 without blaCTX-M were aminoglycoside-susceptible and 3 were ciprofloxacin-susceptible. Sequencing the blaOXA-48-IS1999 links on both sides of blaOXA-48 in 2 isolates showed one to be identical to GenBank AY236073, the other had its upstream IS1999 copy disrupted by IS1. PCR indicated that the other 8 isolates had combinations of both structures, suggesting multiple blaOXA-48 copies.

Conclusion: The association of OXA-48 with multiple other $\beta$-lactamases, in diverse K. pneumoniae strains with different antibiograms indicate that OXA-48-mediated carbapenem resistance in the UK has varied origins. The potential for spread warrants high vigilance to ensure prompt detection and adequate control measures.
carbapenems were isolated from three patients with a later or subsequent colonisation/infection by carbapenem-resistant strain after a long therapy with a carbapenem (multiresistant strains are stored for at least one week in the laboratory). Two isolates produced SHV-5 ESBL, three DHA-1 AmpC, and one both enzymes − SHV-5 and DHA-1. bla genes were carried out on the plasmids with FII and FIC replicons. Only two carbapenem resistant isolates showed the same PFGE pattern, despite the carbapenem susceptible and resistant strains isolated from three patients that showed the same pattern. All resistant isolates did not express neither OmpK35 or OmpK36 porins. The genes encoded these porins were not detected. OmpK37 was identified in four isolates.

Conclusions: This work showed a possible in vivo selection of carbapenem resistant subpopulation in SHV-5 and/or DHA-1 producing K. pneumoniae due to a decreasing permeability of outer membrane. Such studies are important for the verification of possible treatment failure of carbapenems in treatment of infections caused by ESBL/AmpC producers. MLST analysis is ongoing.

This work was partially financed by a research project MSM2E08003 and MSM0021620819.

P1698  In vivo selection of imipenem-resistant Klebsiella pneumoniae producing clavulanic acid inhibited extended-spectrum ß-lactamase and plasmid-encoded cephalosporinase

G. Cazen*, F. Naas, M. Guibert, P. Nordmann (Le Kremlin-Bicêtre, Clamart, FR)

Objectives: Resistance to carbapenems, while uncommon in Enterobacteriacae, can be mediated by two main mechanisms. The first involves production of a chromosomal or plasmid-mediated cephalosporinase combined with decreased permeability due to loss or alteration of porins. The second one is the production of a ß-lactamase that is capable of hydrolysing carbapenems (carbapenemases). This study was aimed to characterise the genetic basis of carbapenem resistance in a multidrug resistant clinical isolate of Klebsiella pneumoniae after prolonged imipenem exposure.

Methods: Four Klebsiella pneumoniae (KP1−4) isolates were recovered sequentially from rectal swabs and clinical samples in an elderly patient. Laboratory investigations included pulsed-field gel electrophoresis of XbaI digested genomic DNA, isoelectric focusing, imipenem hydrolysis, resistance gene analysis by PCR and sequencing, and outer membrane protein analysis by SDS-PAGE. Plasmid analysis by DNA-DNA hybridisation, electroporation and conjugation were also performed.

Results: KP1 to 3 were recovered from urine, blood culture and rectal swab respectively. They showed resistance to all ß-lactams, except carbapenems. KP4 was recovered after 24 days of imipenem therapy from colostomy. It had an identical PFGE pattern when compared with those of KP1 to 3. However, resistance to all carbapenems was present only for KP4. Molecular characterisation revealed that, KP4 expresses the ESBL blaCTX-M-15 gene, plasmid-mediated AmpC ß-lactamase gene (blaDHA-1) and blaTEM-1 gene (existing in KP1−3), and failed to express OmpK36, because of a point mutation leading to a premature stop of the protein. All four isolates carried two large plasmids (65 and 95 kb). DNA-DNA hybridisation revealed that the 95 kb plasmid harboured blaCTXM-15 gene and could be transferred to Escherichia coli by mating out assay. The plasmid-mediated blaDHA-1 ß-lactamase gene was harboured on the 65 kb plasmid but could be transferred by conjugation. No carbapenem-hydrolysing enzymes nor carbapenem-hydrolysis could be identified in that isolate.

Conclusion: The present study demonstrated the development of carbapenem resistance in a KP isolate harbouring ESBL and plasmid-mediated cephalosporinase, after prolonged imipenem exposure. Resistance was related to the loss of OmpK36 expression.

P1699  Failure of meropenem therapy for hospital-acquired multiple-resistant Klebsiella pneumoniae urinary tract infection

A. Hamouda, S. Dancer, S. Amyes* (Edinburgh, Lanarkshire, UK)

Objectives: Carbapenem resistance among the Enterobacteriaceae is of great concern as these compounds are considered the last resort for treating infections caused by these bacteria. The purpose of this study was to investigate carbapenem-resistance mechanism in a Klebsiella pneumoniae collected from urinary tract infections.

Methods: Two K. pneumoniae strains K1 and K2 were collected from urine on 8th and 13th May 2008 respectively from a septic and catheterised 71-year-old man from Hairmyres Hospital, Lanarkshire treated with meropenem. MICs of antibiotics were performed according to the BSAC guidelines. Isolates found resistant to ceftazidime and piperacillin were considered as ESBL producers and were subsequently subjected to confirmatory tests. ESBL production was confirmed by double and combination disk methods. The blaTEM, blaSHV, blaCTX-M, blaOXA, and blakPC and metallo-ß-lactamases genes were screened by PCR and multiplex PCR respectively and confirmed by sequencing. Pulsed-field gel electrophoresis (PFGE) typing was performed using XbaI restriction endonuclease.

Results: K. pneumoniae strain K2 was resistant to all antibiotics tested except colistin. The MICs (mg/L) were: amikacin (>32), gentamicin (>32), tobramycin (>32), ampicillin (>64), ceftazidime (>256), cefepime (>128), imipenem (4), meropenem (8), piperacillin (>64), piperacillin/tazobactam (>64), sulbactam (>2), ceftaxime/clonamycin (>256), colistin (0.5), ciprofloxacin (>8), minocycline (32) and tigecycline (2). K. pneumoniae strain K1 was resistant to meropenem (16) but susceptible to imipenem and meropenem with MICs of 1 and 0.25 mg/L respectively. ESBL confirmatory tests were positive for both isolates and the presence of blaTEM-1, blaSHV-1, blaCTX-M-15, blaOXA-1 was confirmed by DNA sequencing analysis. No metallo-ß-lactamases nor KPC ß-lactamases were found. The two K. pneumoniae strains were considered to belong to the same PFGE type.

Conclusion: A case of failed meropenem treatment for hospital-acquired multiply-resistant Klebsiella pneumoniae urinary tract infections resistant to carbapenems producing CTX-M-15 is reported. Resistance to all major antibiotics including tigecycline is consistent with permeability changes including porin loss and upregulated efflux. No carbapenemase could be detected.

P1700  Multifocal emergence of ESBL-producing Klebsiella pneumoniae clone with differential non-carbapenemase-mediated resistance to carbapenems in Italian hospitals


Background: Klebsiella pneumoniae is a major nosocomial pathogen. Carbapenems are among the few active drugs against multidrug-resistant (MDR) strains of K. pneumoniae producing extended-spectrum ß-lactamases (ESBLs). Carbapenem resistance is increasingly reported in this species, being a matter of great clinical concern. In this work we report on the recent multifocal emergence, in several Italian hospitals, of an ESBL-producing K. pneumoniae clone with differential non-carbapenemase-mediated resistance to carbapenems.

Methods: Antimicrobial susceptibility was determined by disk diffusion and Etest. Clonal relatedness was investigated by PFGE of XbaI-digested genomic DNA. Carbapenemase activity was assayed spectrophotometrically. ß-lactamase genes were investigated by PCR and sequencing. Outer membrane proteins (OMPs) were investigated by SDS-PAGE and by analysis of the corresponding genes.

Results: during 2007−08, ESBL-positive K. pneumoniae isolates showing a differential carbapenem resistance phenotype (resistance to ertapenem, reduced susceptibility to meropenem and susceptibility to imipenem) started to be reported from several Italian hospitals. We have
investigated 22 nonreplicate isolates showing this phenotype from 6 different hospitals located in various Italian regions. Isolates were from bloodstream, lower respiratory tract, urinary tract and intra-abdominal infections. Carbapenemase activity and known carbapenemase genes were not detectable. All isolates produced the CTX-M-15 ESBL and resident SHV enzyme. Decreased expression of the k36 OMP was detected in all isolates. PA[60(N(40 mg/L)] did not affect carbapenem MICs. PFGE analysis revealed a dominant clone (20 isolates) present in the 6 hospitals, plus one additional unrelated clone from a single hospital.

**Conclusions:** Although production of plasmid-encoded carbapenemases is the most common mechanism of carbapenem resistance in *K. pneumoniae*, strains with non-carbapenemase-mediated resistance have occasionally been described. To our best knowledge, this is the first evidence for multifocal clonal spread of ESBL-producing *K. pneumoniae* with a similar resistance phenotype. Clinical impact, and implications for detection and reporting by the clinical microbiology laboratory are discussed.

**References:**

[1] The presence of a 45Kda protein OmA, a 25Kda alkali-inducible disulfide interchange protein, the putative toluse tolerance protein, a putative outer membrane protein, a putative exported protein and the chaperone GroEL in all the strains. 2. In the community-acquired strains, which were susceptible to aztreonam and piperacillin, the protein CarO was found and it is important to highlight a putative outer membrane protein (circa 35 Kda), which was not found in the isolates causing nosocomial infections, and 3. In the carbapenem resistant strains the outer membrane protein HMP, three putative outer membrane proteins and the β-lactamases class C and OXA-26.

**Objectives:** To investigate the differences in antimicrobial susceptibility and clonal relatedness against carbapenem-resistant *Acinetobacter baumannii* collected in several Spanish Hospitals in two different time periods (from 15 April-1998 to 17 November-1998 and from January to February 2006).

**Methods:** A total of 93 imipenem-resistant *A. baumannii* were collected from 25 general hospitals in Spain in 1998 (44 isolates) and 2006 (49 isolates). Species identification and antibiotic susceptibilities were determined by Vitek-2. MICs were determined by microdilution, agar dilution methods and E-test (tigecycline and colistin). Clonal relatedness was determined by RAPD-PCR and ERIC2-PCR. E-test with imipenem (IMP) and imipenem plus EDTA was performed to check metallo-β-lactamase (MBL) production. The isolates were screened by PCR analysis with specific primers for carbapenemase genes.

**Results:** Epidemiologic relatedness of 44 imipenem-resistant isolates from 1998, revealed only 4 different genetic backgrounds. In the isolates from the second period, the four original clones were maintained and 4 new profiles, closely related with previous ones, were identified. Overall, 100% *A. baumannii* isolates were resistant to >3 antimicrobials classes. All the isolates carried a gene encoding a β-lactamase belonging to OXA-51-like group. 15 of the 44 carbapenem-resistant (34.1%) from 1998 and 13 of the 49 isolates (26.5%) from 2006 contained the insertion sequence ISAba1 upstream the promoter region of the blaOXA-51-like gene. 66% of the isolates from 1998 carried a gene encoding an OXA-40-like enzyme, alone (21 isolates) or combined with an IMP carbapenemase (8 isolates). In 2006, OXA-40-like oxacillinase was the enzyme prevalent (22 isolates; 18 isolates alone, 4 combined with another enzyme) but OXA-58-like and OXA-23-like enzymes were detected in a significant percentage of isolates (28.6% and 18.4% respectively). Some degree of heterogeneity was observed within each genetic background. This heterogeneity was higher in groups from 2006 than in groups from 1998.

**Conclusions:** Several clones among *A. baumannii* multiresistant isolates are maintained over the time in Spanish hospitals. The predominant clones appeared in most than one hospital indicating possible inter-hospital spread of carbapenem resistant *A. baumannii* strains. OXA-40-like enzyme, is the enzyme prevalent at Spanish hospitals.
**P1704** Spread of different STs of *Acinetobacter baumannii* carrying specific acquired carbapenem-hydrolysing oxacillinases

F. Grosso*, S. Quinteira, L. Peixe (Porto, Famalicao, PT)

**Objectives:** OXA-40 was the only acquired carbapenem-hydrolysing oxacillinase detected in *Acinetobacter baumannii* (AB) for several years in our country, but since 2006 we observed an increase in the appearance of OXA-23-producers, some of them associated to outbreaks. Recently, some isolates carrying the blaOXA-58 were also identified. Together they greatly contribute to the high rate of imipenem-resistance in this species in our hospitals. The aim of this work was to investigate the relationship between blaOXA-23, blaOXA-58 and blaOXA-40-carrying isolates by PFGE and two different schemes of MLST, comparing their capacity of discrimination.

**Methods:** We analysed the population structure of eight isolates representative of blaOXA-23 (n=2), blaOXA-40 (n=4) and blaOXA-58 (n=1) strains, disseminated and persisting over years, that were selected among well characterised 186 AB isolates recovered from 3 Portuguese hospitals. A carbapenem-susceptible isolate (CSAB) was also included. Isolates were identified by API20GN and by 16S rRNA sequencing. Susceptibility testing (CLSI) and PFGE (APaI) were performed. blaOXA-23, blaOXA-40, blaOXA-51-like and blaOXA-58 genes were identified by sequencing of respective amplicons. MLST schemes were performed according to Bartual et al. (2005) (B) and Brisse et al (www.pasteur.fr/mlst) (IP).

**Results:** A clonal relatedness was observed by PFGE for all isolates except for the one carrying blaOXA-58. The sequence analysis of blaOXA51-like revealed the presence of OXA-66 in all isolates, except for the blaOXA-58-carrying isolate. MLST typing revealed that this isolate belonged to a new ST by the B-scheme and to ST15 by IP-scheme. Both blaOXA-23-carrying AB and CSAB belonged to the disseminated ST22 (B-scheme) and to ST2 (IP-scheme). According to B-scheme, the OXA-40-producing AB isolates corresponded to a new ST, which is a DLV of ST22, whereas with MLST-IP scheme we observed that they shared 6 of the 7 alleles of ST2.

**Conclusions:** In our country, carbapenem-resistant AB are mainly related to the dissemination of three different STs, each one associated to different acquired OXA-type. The rapid increase in the recently described OXA-23 producers is associated with the spread of the internationally disseminated ST22 AB clone. Remarkably, MLST data indicates a common origin between ST22 and the persistent new ST OXA-40-producing clone.

**P1705** Characterisation of blaOXA-40-carrying plasmids among *Acinetobacter* spp. isolates in Portuguese hospitals

F. Grosso*, S. Quinteira, L. Peixe (Porto, Famalicao, PT)

**Objectives:** OXA-40, an acquired carbapenemase initially confined to Iberian Peninsula and associated to a long term prevalent *Acinetobacter baumannii* (Ab) strain, has been more recently observed in Ab outbreaks in USA and in *A. haemolyticus* (Ah) in Portugal. The purpose of this study was to characterise the genetic background of this carbapenemase.

**Methods:** Thirty OXA-40-Ab-producers were selected from a collection obtained during 2001–2007 in 2 Portuguese hospitals. An OXA-40-producing Ab and one imipenem-susceptible Ab (ISAb) isolate that did not present any acquired oxacillinase, were also included. Chromosomal and/or plasmid location of blaOXA-40 was determined by I-CASt hybridisation with a specific probe for this oxacillinase and for 16S rDNA. Plasmid extraction followed by sequencing was performed in order to assess the vicinity of the blaOXA-40 gene. PCR for repAc1 was also conducted. The amplicons obtained were used as a probe for subsequent I-CEul hybridisation.

**Results:** All OXA-40 producers revealed a plasmid location for blaOXA-40. Plasmids sizes varied from 45 kb to 220 kb. In some isolates was also observed a chromosomal hybridisation with blaOXA-40 probe. All plasmids showed identical regions upstream the blaOXA-40 gene which presented homology with a plasmid previously described (pAB02). The Ab plasmid also presented identity with the pAB02 plasmid in the downstream region of blaOXA-40 gene. However, the Ab isolates displayed homology with other plasmids (pAB2, p2ABAYE, pAC1CU1) in the regions following the end of the blaOXA-40 gene. The ISAb revealed a plasmid with ca. 60 Kb, that presented the repAc1 gene, characteristic of the downstream regions of blaOXA-40 in Ab plasmids. Duplicated motifs associated to insertion process were not observed in flanking regions of blaOXA-40.

**Conclusions:** The blaOXA-40 seems to have been acquired by particular disseminated plasmids with repAC1 in Ab isolates persistently recovered from our hospitals, and was co-located in the chromosome in most of them. The persistence of these plasmids among isolates of the Iberian clone might play a role in the maintenance of this successful lineage. The Ab isolate carried other OXA-40 plasmid similar to the pAB02 plasmid, which enable us to anticipate its dissemination among *Acinetobacter* species. An insertional process does not seem the most reliable explanation for the plasmidic acquisition of this carbapenemase gene, as already described for other oxacillinases.

**P1706** Clonal outbreak of multidrug-resistant ST22 *Acinetobacter baumannii* in a university hospital, Plzen, Czech Republic

F. Grosso*, M. Pinheiro, S. Quinteira, T. Bergerová, J. Hrabáč, L. Peixe (Porto, Famalicao, PT; Plzen, CZ)

**Objectives:** *Acinetobacter baumannii* has recently emerged as a pathogen of substantial concern namely due to the acquisition of resistance to several antibiotics, being associated to a considerable mortality. Since December 2007 a dramatic increase in infections caused by MDR *A. baumannii* (MDR Ab) was observed in different units of the University Hospital of Plzen. The objective of this study was to investigate this outbreak using epidemiologic and molecular techniques and to relate it to previous reports of MDR Ab.

**Methods:** In the beginning of 2008, 1-month survey of carbapenem resistant strains of *A. baumannii* was performed. From a total of 97 isolates collected, nine non-repetitive isolates causing infection were selected from different units involved in the outbreak – Cardiosurgery (n = 5), Surgery (n = 3) and Pulmonary (n = 1). MICs to several antibiotics were determined by standard microdilution broth method according to EUCAST recommendation. Carbapenemase activity was determined by a bioassay. The OXA-51 type was determined by PCR followed by sequencing. ISAb1 upstream the blaOXA-51 and acquired OXA-type β-lactamases (blaOXA-23-like, blaOXA-40 and blaOXA-58-like) were searched by a multiplex PCR. The isolates were typed by PFGE with digestion with Apa I endonuclease and by MLST scheme of Bartual et al. (2005).

**Results:** Isolates were resistant to ciprofloxacin and to β-lactams, including meropenem, with the exception of one isolate susceptible to imipenem. Most isolates were susceptible to aminoglycosides, with the exception of gentamicin. Susceptibility to colistin was observed only in three isolates. No carbapenemase activity was found as well as acquired carbapenemases. ISAb1 was found upstream of OXA-66 in all isolates. PFGE indicated a common pattern, identified as belonging to ST22 by MLST.

**Conclusions:** Spread of ST22 *A. baumannii* strain, the main clone in Czech Republic, and also globally disseminated, was responsible for the emergence of MDR Ab in this hospital. Particular features of this lineage might contribute for its success and prevalence.

**P1707** Identification and typing of multiresistant *Acinetobacter baumannii* in Latvian hospitals

A. Balode*, M. Petrova, D. Berzina, J. Denisova, U. Dampis, J. Zestkova, E. Mikliaevica (Riga, LV)

**Objectives:** *Acinetobacter baumannii* is an important nosocomial pathogen which is often associated with multiple drug resistance. First imipenem resistant *Acinetobacter baumannii* isolates in Latvian hospitals were detected in fall of 2007 and afterwards rapidly spread
Detection of the OXA-58 carbapenemase in clinical isolates of Acinetobacter baumannii from Cochabamba, Bolivia


Objectives: The aim of this work was to study the presence of carbapenemases and their related genetic structures in clinical isolates of A. baumannii from hospitals in Cochabamba, Bolivia.

Methods: The study included 10 A. baumannii isolates obtained in a hospital from Cochabamba, Bolivia (Hospital Gastroenterológico Boliviano-Japonés) during 2008. Susceptibility to antimicrobial agents was determined by disk diffusion method following the CLSI recommendations. Antibiotics tested were amikacin, ciprofloxacin, ceftazidime, cefepime, cefotaxime, cefoxime, and levofloxacin. Clonal relatedness was performed by PCR-fingertyping, multiplex-PCR and sequencing analysis showed the presence of the OXA-51 like element and 103 isolates also contained ISAba 3 and two were positive for ISAba 1. Multiplex-PCR and sequencing experiments with the corresponding primers, (integrons were also investigated by multiplex PCR. Strains were molecularly typed by rep-PCR using the DiversiLab system. Plasmids were isolated to determine if imipenem-resistance was transferable. Expression of the adeB and adeJ efflux pump genes was performed by semi-quantitative real-time RT-PCR.

Results: Molecular typing revealed that the patient was infected with the same A. baumannii strain during the 2-month period. All isolates were resistant to imipenem, meropenem, cephalosporins, fosfomycin, gentamicin, ampicillin-sulbactam, beta-lactams, piperacillin and piperacillin-tazobactam and sensitive to tobramycin. Isolates were positive for blaOXA-58-like gene. Imipenem-resistance was not transferable. The patient was treated with a combination of amikacin, ciprofloxacin and co-trimoxazole for an unrelated bacterial infection. After treatment, all subsequent isolates were resistant to tigecycline and co-trimoxazole and recorded higher levofloxacin MICs simultaneously (see Table).

Conclusions: These data show the propensity of A. baumannii to develop efflux-mediated resistance during antimicrobial therapy. This efflux-mediated resistance was stable and had a broad specificity.

P1709 Development of resistance to tigecycline, co-trimoxazole and tobramycin associated with increased expression of the Acinetobacter baumannii efflux pump AdeB in a hospitalised patient

PG. Higgins*, H. Seifert (Cologne, DE)

Objectives: To investigate the mechanism of stepwise acquired multidrug resistance in Acinetobacter baumannii isolated from a hospitalised patient.

Methods: 13 consecutive multi-drug resistant A. baumannii isolates were recovered from the same patient during a 2-month period. Sensitivity to antimicrobials was performed by VITEK2, broth microdilution and E-test. Speciation was confirmed by gyrB multiplex. Presence of OXA-type enzymes were investigated by multiplex PCR. Strains were molecularly typed by rep-PCR using the DiversiLab system. Plasmids were isolated to determine if imipenem-resistance was transferable. Expression of the adeB and adeJ efflux pump genes was performed by semi-quantitative real-time RT-PCR.

Results: Molecular typing revealed that the patient was infected with the same A. baumannii strain during the 2-month period. All isolates were resistant to imipenem, meropenem, cephalosporins, fosfomycin, gentamicin, ampicillin-sulbactam, beta-lactams, piperacillin and piperacillin-tazobactam and sensitive to tobramycin. Isolates were positive for blaOXA-58-like gene. Imipenem-resistance was not transferable. The patient was treated with a combination of amikacin, ciprofloxacin and co-trimoxazole for an unrelated bacterial infection. After treatment, all subsequent isolates were resistant to tigecycline and co-trimoxazole and recorded higher levofloxacin MICs simultaneously (see Table).

Conclusions: These data show the propensity of A. baumannii to develop efflux-mediated resistance during antimicrobial therapy. This efflux-mediated resistance was stable and had a broad specificity.

P1710 Tigecycline resistance of Acinetobacter bloodstream isolates in a teaching hospital

S. Vamucacopoulou*, K. Michalacopoulou, C. Bartazali, I. Spilopoulou, E.D. Anastassiosis, M. Christofolou (Patras, GR)

Objective: Acinetobacter calcoaceticus-A. baumannii complex, an opportunistic pathogen frequently involved in infection outbreaks in Intensive Care Units (ICU), constitutes a serious problem because of its virulence and multidrug resistance. Tigecycline (TIG), is a glycyclcline antibiotic with broad-spectrum activity that appears to be the only new
Genetic basis of resistance to aminoglycosides in Acinetobacter baumannii
Outbreak of pan aminoglycoside-resistant armA producing A. baumannii were detected. armA (resistant to four or more used antimicrobials) in our hospital. As MBL was produced by many MDR isolates, imipenem seems not to be useful in empiric therapy of critically ill, bacteremic patients. Colistin and tigecycline remain the only active agents towards such strains, according to our results.

Methods: A total of 250 consecutive isolates were collected from infected and colonised patients in 8 major hospitals in Kuwait. They were identified by VITEK-2 and their susceptibility to 18 antibiotics determined by Etest method. Susceptibility and resistance were assessed according to the recommended criteria of the CLSI and FDA for tigecycline. Metallo-β-lactamase (MBL) production was detected by the MBL Etest method.

Results: Resistance to many of the tested antibiotics were very high, in particular to amikacin (82.8%), gentamicin (68.4%), cefepime (69.6%), ciprofloxacin (73.2%) and piperacillin-tazobactam (71.2%). It is noteworthy that, in this study, resistance to tigecycline was as high as 13.6%. Similarly, of clinical importance were the resistance rates of 25.2, 37.3 and 12% to imipenem, meropenem and colistin, respectively. The overall prevalence of MBL was 37.2%. Both MIC50 and MIC90 of amikacin, ceftazidime, cefepime and piperacillin-tazobactam were each 256 µg/ml, while MIC50 and MIC90 of imipenem, meropenem, tigecycline and colistin were 1.5 and 32, 3 and 32, 0.38 and 3, and 1 and 3, respectively. Interestingly, only 36% of MBL-positive strains were from infected patients, with the highest rate (48.3%) from Mubarak hospital followed by 44.9% from Al-Jahra hospital.

Conclusion: Multi-resistant Acinetobacter spp is highly prevalent in our hospitals and has reached an alarming level which justifies continuous surveillance and stringent infection control measures to control its spread.

P1713 Outbreak of pan aminoglycoside-resistant armA producing Acinetobacter baumannii in a Korean hospital

Background: Recently, 16S rRNA methylase-mediated highly aminoglycoside-resistant isolates of Acinetobacter baumannii have been reported worldwide. However, there has been only one case report of outbreak by pan aminoglycoside resistant armA producing A. baumannii so far. We prospectively investigate a nosocomial outbreak of infection with pan aminoglycoside resistant A. baumannii in neonatal ICU and respiratory ward of Korean hospital.

Methods: Prospective outbreak investigation was done in a tertiary 1,000-bed university hospital in Korea. Antibiograms determined by the double-disk diffusion technique. All isolates were screened for the presence of aminoglycoside modifying enzymes, class 1 integron, and 16S rRNA methylases (rmtA, rmtB, rmtC and armA) genes by PCR and sequencing.

Results: Nine isolates from eight patients were investigated for three months. All A. baumannii isolates shown resistant to piperacillin, piperacillin/tazobactam and all aminoglycosides tested except neomycin. Four strains were resistant to meropenem, 2 intermediate, and 3 sensitive in disk diffusion test. PCR and sequencing showed that all isolates were negative for aac(3)-1, aadB, aph(3’)-2, aph(3’)-3, aph(3’)-6, aprA-1, aprA-2, aac(3)-2, aac(3)-3, aac(3)-4, aac(6’)-2b, aph2, rmtA, rmtB, rmtC, and rmtD. Positive for aac(6’)-1b, int 1, orf513 and armA. Molecular typing showed two types of closely related strains.

Conclusion: Outbreak of pan-aminoglycosic resistant A. baumannii is becoming a great concern about nosocomial infection control in Korean hospital. Appropriate antibiotics usage, management of active surveillance system, and strict contact precaution should be kept to prevent further occurring of outbreak.

P1714 Mechanisms of resistance to ciprofloxacin, ampicillin/sulbactam and imipenem in Acinetobacter baumannii in Taiwan

Objectives: Nosocomial infections caused by multidrug-resistant Acinetobacter baumannii (MDRAB) have been increasing in recent years, posing serious threat to public health worldwide.

Antibiotic resistance pattern of Acinetobacter species in Kuwait hospitals
N. Al-Sweth*, M. Al-Hubali, V. Rottimi (Kuwait, KW)

Objective: As high and increasing resistance of Acinetobacter spp to many potential therapeutic agents limit the choice of appropriate therapy, this study was carried out to determine the resistance pattern of strains causing clinically proven infections and those associated with mere colonisation.

P1711 Genetic basis of resistance to aminoglycosides in Acinetobacter species and spread of armA in Acinetobacter baumannii sequence group 1 in Korean hospitals

A total of 75 Acinetobacter isolates resistant to all available aminoglycosides obtained from two Korean hospitals were studied for the genetic basis of resistance to aminoglycosides. Minimal inhibitory concentrations of aminoglycosides were higher in A. baumannii isolates (n = 61) than Acinetobacter genomospecies 3TU isolates (n = 14).

Genes encoding aminoglycoside-modifying enzymes, ant(3’)-Ia, aac(6’)-Ib, aph(3’)-Ia, aac(3)-Ia and aph(3’)-VI and 16S rRNA methylase armA were detected. ant(3’)-Ia and aac(6’)-Ib were commonly detected in both Acinetobacter species, but armA and aph(3’)-Ia were only detected in A. baumannii. armA was located on the plasmids. A. baumannii isolates carrying armA were classified into seven pulsotypes, but belonged to sequence group 1. The combination of aminoglycoside-modifying enzymes is responsible for the moderate-level resistance to aminoglycosides in Acinetobacter genomospecies 3TU, whereas armA is responsible for the high-level resistance to aminoglycosides in A. baumannii sequence group 1.

therapeutic option for serious infections caused by multidrug resistant (MDR) Gram negative bacteria. The aim of this study was to define the susceptibility profile of bloodstream Acinetobacter isolates to antibiotics, including TIG, during the last three years in Patras University Hospital.

Methods: A total of 162 Acinetobacter bloodstream isolates were collected between September 2005 to September 2008 from inpatients hospitalised in ICU (82), in Internal Medicine units (52) and in Surgical Wards (28). Identification at species level was performed using Gram negative BD BBL Crystal ID system. Antimicrobial susceptibility was tested by disk diffusion method, according to CLSI criteria, for cefepime (FEP), cefazidime (CAZ), imipenem (IMP), ertapenem (AZT), gentamicin (GM), netilmicin (NET), amikacin (AN) and ciprofloxacin (CIP), and by E-test strips (AB Biodisk) for MIC of colistin (CL) and TIG. MIC breakpoint of susceptibility for CL is equal or less than 1 microg/mL whereas for TIG is equal or less than 2 microg/mL. IMP-resistant isolates were examined by double E-Test strips (IMP versus IMP plus EDTA) (AB Biodisk) for detection of metallo-β-lactamases (MBL).

Results: Forty five isolates were recovered during the first year, 61 the following year, whereas 56 isolates were identified the third year, one isolate per patient. A total of 159 (98%) isolates were identified as Acinetobacter baumannii. Resistance rate was as high as 96%, 94%, 93% and 90% to AZT, IMP FEP and CAZ, respectively, and 87%, 86%, 85% and 68% to AN, NET, CIP and GM. Among IMP-resistant isolates, 95% were MBL (+). No isolate was resistant to CL as MIC was 0.38–1 microg/mL. All isolates were susceptible to TIG as MIC ranged between 1–2 microg/mL.

Conclusions: A total of 148 (86%) Acinetobacter isolates were MDR (resistant to four or more used antimicrobials) in our hospital. As MBL was produced by many MDR isolates, imipenem seems not to be useful in empiric therapy of critically ill, bacteremic patients. Colistin and tigecycline remain the only active agents towards such strains, according to our results.
**Objective:**
The study included a total of sixty-eight isolates of Acinetobacter baumannii: resistant strains except one. The imipenem MIC among blaoxa-23-like positive isolates was 38% in the global sample and 50% (38 out of 77) in patients who had previous admission at surgical-ICU and 48% at general ICU (mean stay of 27.5 days). Most frequent underlying conditions were high blood pressure (42%), heart disease (32%), diabetes (30%), pulmonary disease (22%), cancer (14%) and renal failure (14%). Most frequent predisposing factors were previous surgery (83%), central venous catheter (88%), urinary catheter (92%), mechanical ventilation (84%), tracheostomy (54%), parenteral nutrition (54%) and blood transfusions (48%). 41% patients had received previous carbapenem treatment; 42% piperacillin-tazobactam and 17% another antibiotic. The source of MRAB was respiratory (46%), abdominal (14%) and surgical wound in 20% (not clarified in 5%); 42 patients developed complications. All A. baumannii isolates were carbapenem, cephalosporins, piperacillin-tazobactam and quinolones resistant; 26% were ampicillin-sulbactam susceptible, 38% amnoglycoside, colistin and tigecycline susceptible but ampicillin-resistant; 26% were ampicillin-sulbactam susceptible, 38% amnoglycoside, colistin and tigecycline susceptible but ampicillin-sulbactam resistant and 20% only colistin susceptible.

**Materials and Methods:**
A total of 997 consecutive non-duplicate MRAB isolates were studied (35 female). 24 (24%) were considered as colonisation; 25 developed bacteraemia and 57 non-bacteraemic infection. Mean age was 56 years (range 18–88); 16% patients were located in Medical Wards and 84% in Surgical Wards; 33% had a previous admission at surgical-ICU and 48% at general ICU (mean stay of 27.5 days). Most frequent underlying conditions were high blood pressure (42%), heart disease (32%), diabetes (30%), pulmonary disease (22%), cancer (14%) and renal failure (14%). Most frequent predisposing factors were previous surgery (83%), central venous catheter (88%), urinary catheter (92%), mechanical ventilation (84%), tracheostomy (54%), parenteral nutrition (54%) and blood transfusions (48%). 41% patients had received previous carbapenem treatment; 42% piperacillin-tazobactam and 17% another antibiotic. The source of MRAB was respiratory (46%), abdominal (14%) and surgical wound in 20% (not clarified in 5%); 42 patients developed complications. All A. baumannii isolates were carbapenem, cephalosporins, piperacillin-tazobactam and quinolones resistant; 26% were ampicillin-sulbactam susceptible, 38% amnoglycoside, colistin and tigecycline susceptible but ampicillin-sulbactam resistant and 20% only colistin susceptible. Empirical antibiotic treatment was wrong in 90% and mortality rate was 38% in the global sample and 50% (38 out of 77) in patients who developed MRAB infection.

**Conclusions:** Knowledge about risk factors and clinical aspects of MRAB is necessary to improve empirical treatments and decreased associated mortality and morbidity.

**Methods:**
The susceptibility to 9 antimicrobial agents of 35 clinical Acinetobacter baumannii isolates from Chang Gung Memorial Hospital was tested. Isolates were examined by PCR and sequencing for β-lactamase genes and mutations of gyrA and parC genes. The expression of AdeB, an efflux pump protein, was evaluated by real-time quantitative PCR.

**Results:**
The level of adeB expression correlated with resistance to ciprofloxacin and ampicillin/sulbactam in Acinetobacter baumannii isolates. Furthermore, mutation analyses of gyrA and parC showed that certain mutations in these target genes, together with over-expression of Ade transporter, conferred the resistance to ciprofloxacin. All 13 isolates with full resistance to ciprofloxacin had both high adeB expression and mutations in gyrA or parC, but 4 intermediately resistant isolates had only high adeB expression without mutations in gyrA or parC, in contrast to 18 susceptible isolates with low adeB expression and without any mutations. Two ciprofloxacin-resistant isolates in our study had mutations only at parC but not at gyrA, suggesting parC might not only be a minor or secondary target for the action of quinolones in Acinetobacter baumannii. Sixteen isolates (45.7%) carrying type 1 integron were multi-drug resistant (MDR) and more resistant to imipenem, amikacin, gentamicin, ceftazidime, cefepime, or piperacillin than those without the integron (all p < 0.002). The type 1 integron contained different resistance gene cassettes, including 5′S-blaIMP-1-aadA4–3′CS, 5′S-aacA4-2Km2-3′CS, and 5′S-aacC1-3′aadA1-3′CS.

**Conclusion:**
The expression of adeB gene was associated with resistance to ciprofloxacin and ampicillin/sulbactam in Acinetobacter baumannii. Multiple mutations in gyrA and parC also played a role in ciprofloxacin resistance. The major metallo-β-lactamase contributing to imipenem resistance in Acinetobacter baumannii in Taiwan was blalmp-1, which was carried by class 1 integron. Class 1 integron was associated with MDR phenotype to Acinetobacter baumannii.
Results: The antimicrobial susceptibility of Acinetobacter isolates is summarised in the Table.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC, μg/ml</th>
<th>% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Imipenem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997–1999</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>2002–2004</td>
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<td>2</td>
</tr>
<tr>
<td>2006–2007</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Amikacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997–1999</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>2002–2004</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>2006–2007</td>
<td>256</td>
<td>≥512</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997–1999</td>
<td>32</td>
<td>≥256</td>
</tr>
<tr>
<td>2002–2004</td>
<td>128</td>
<td>≥256</td>
</tr>
<tr>
<td>2006–2007</td>
<td>128</td>
<td>≥256</td>
</tr>
<tr>
<td>Netilmicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997–1999</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2002–2004</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2006–2007</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td></td>
</tr>
<tr>
<td>1997–1999</td>
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<td>32</td>
</tr>
<tr>
<td>2002–2004</td>
<td>64</td>
<td>≥128</td>
</tr>
<tr>
<td>2006–2007</td>
<td>≥128</td>
<td>≥128</td>
</tr>
<tr>
<td>Colistin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997–1999</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2002–2004</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2006–2007</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

ND, Not Determined.

A constant increase in resistance rates to ciprofloxacin (from 31.0% to 91.5%), gentamicin (from 69.0% to 84.2%), and amikacin (from 9.4% to 79.1%) was observed from 1997/99 to 2006/07. In the same time, the percentage of isolates non-susceptible to imipenem remained relatively low: 3.0% in 1997/99, 6.1% in 2002/04, and 4.5% in 2006/07. Netilmicin was the most active in vitro among aminoglycosides (21.8% non-susceptible isolates) and colistin was the most active among all antibiotics tested (no resistant isolates) in 2006/07.

Conclusions: The rapid increase in antimicrobial resistance, particularly to ciprofloxacin and amikacin, in nosocomial strains of Acinetobacter spp. in Russia is noteworthy. Based on results of in vitro surveillance studies imipenem, netilmicin and colistin may be recommended as effective treatment for Acinetobacter infections.

Results: According to the analysis of the surveillance study, Imipenem non-susceptibility has had a 3.1 fold (2.8–3.4) increase from 12.6% in 2002 to 40.1% in 2007. Subjects <18 years old had significantly lower rates (8.8 and 31.1% for 2002 and 2007) than adults and the elderly (p < 0.001), but with similar trends. There were significant differences by source, with blood isolates at 34.8%, and sputum at 43.2% in 2007. The predictions for imipenem non-susceptibility for 2010 according to the mathematical model are: overall rate 62.5% (60.6–64.3); <18 years 53.2% (50.9–55.5); 65 and older 68.5% (66.6–70.3); blood/CSF 57.4% (55.1–59.7); sputum 65.7% (63.8–67.6). Multiple resistance was high in 2002 (49.9%), and has increased slowly to 54.9% in 2007. The rate of increase has been steeper in those less than 18 years old than in the elderly (p < 0.001 for interaction), although the latter had the highest rates in 2007 (64.3%). Predictions of multiple resistance according to the mathematical model for 2010 are: overall 58% (56.4–59.6); 65 and older 67.5% (65.9–69); blood/CSF 47.1% (45.3–49); sputum 63.8% (62.2–65.4).

Conclusion: In addition to the predictions of the nonlinear model, the mathematical model showed that reducing the rate of carbapenem use may not reduce resistance to imipenem at the hospital, that reducing the use of quinolones may stop the growth of multiple resistance, but not reduce it, and that reducing transmission at the hospital through interventions may reduce imipenem and multiple resistance to a level similar that strains outside the hospital have.

A. baumannii non-susceptibility.

**P1719** *Acinetobacter infection in adult critical care unit*  
N. Arora*, A. Gogia, P.K. Aggarwal, C. Wattal, B.K. Rao (New Delhi, IN)

**Objective:** To study *Acinetobacter* infection in an adult critical care unit, the sites of infections, predisposing factors/associated conditions and antibiotic sensitivity patterns and to monitor the clinical progress and outcome in these patients.

**Methods:** 40 patients admitted to the adult critical care units who develop *Acinetobacter* infection were analyzed. All relevant clinical specimens were evaluated including blood, endotracheal aspirate, pus, wound swab, CSF and other body fluids, etc. The following variables were analyzed as patient’s age, sex, and the presence of underlying diseases or conditions, number of days in the ICU/HDU, antibiotic therapy and the outcome. *Acinetobacter* isolates were identified phenotypically in the microbiology laboratory, using standard techniques for identification and susceptibility testing. The data was analyzed using standard statistical methods.

**Results:** A total of 40 patients with culture positive *Acinetobacter baumannii* were studied. There were 28 males and 12 females. Mean age of the patients was 55.12 years. The mean duration of ICU stay prior to culture positivity was 10.68 days. Out of 40 patients, all were colistin sensitive while 5 were sensitive to Tigecycline and 15 were moderately sensitive to Tigecycline, 4 were sensitive to Ceftazidime and 10 were moderately sensitive to Cefoperazone-sulbactum. All of them were resistant to imipenem. In 31 cases, endotracheal aspirates were positive, blood culture was positive in 3 patients, sputum was positive in...
4 patients and Urine culture was positive in 3 patients. Out of 40 patients, 24 patients died despite therapy with sensitive antibiotics. 16 patients who grew Acinetobacter survived. Of the 24 patients who died 10 had acute renal failure, 1 had malignancy, 3 had COPD, 4 had Diabetes mellitus, hepatic failure was present in 3 patients and 1 patient was on long term steroids. 16 patients who survived had diabetes mellitus in 4 patients, 3 had hepatic failure and only 1 was on long term steroids.

Conclusion: Most of the patients of Acinetobacter baumannii isolates were multi-drug resistant in our set up and infections due to them were associated with significant mortality. Infection with the resistant strains were associated with prolonged ICU stay, use of indiscriminate broad spectrum antibiotics prior to admission in ICU and multiple co-morbidities like acute renal failure, diabetes mellitus, malignancy and prolonged steroid use.

Bacterial epidemiology

Objective: To evaluate the prevalence and antimicrobial susceptibility pattern (S) of MRSA and vancomycin-resistant E. faecium (VREFM) in four European hospitals. These organisms are usually multi-drug-resistant (MDR) with very limited therapeutic options.

Methods: Non-duplicate consecutive strains causing BSI were collected in the 2005–2008 period from 27 hospitals located in 11 European countries, Turkey and Israel. A total of 22,712 organisms (9,299 Gram-positives [GP]) were collected, including 3,962 S. aureus and 1,700 Enterococcus spp. (677 E. faecium), and tested for S by CLSI broth microdilution methods in cation-adjusted Mueller-Hinton broth.

Results: Overall MRSA rates decreased from 31.5% in 2005 to 23.9% in 2008. Marked decreases occurred in Turkey (from 41.0% in 2005 to 16.2% in 2008), Italy (51.1% to 29.6%), UK (52.3% to 30.9%) and Poland (25.3% to 15.4%). MRSA rates varied widely among the countries evaluated and, in 2008 were highest in Israel (50.0%) and Ireland (44.6%), and lowest in Sweden (1.8%) and Poland (25.3 to 15.4%). MRSArates varied widely among the countries, Turkey and Israel. A total of 22,712 organisms (9,299 Gram-positives [GP]) were collected, including 3,962 S. aureus and 1,700 Enterococcus spp. (677 E. faecium), and tested for S by CLSI broth microdilution methods in cation-adjusted Mueller-Hinton broth.

Results: Overall MRSA rates decreased from 31.5% in 2005 to 23.9% in 2008. Marked decreases occurred in Turkey (from 41.0% in 2005 to 16.2% in 2008), Italy (51.1% to 29.6%), UK (52.3% to 30.9%) and Poland (25.3% to 15.4%). MRSA rates varied widely among the countries evaluated and, in 2008 were highest in Israel (50.0%) and Ireland (44.6%), and lowest in Sweden (1.8%) and Poland (25.3 to 15.4%). Overall VREFM increased from 6.1% (2005) to 10.0% (2008), but occurred only in 5 countries in 2008, varying from 4.6% in Italy and 5.3% in UK to as high as 26.2% in Germany and 22.2% in Ireland. The only country with a consistent VREFM increase was Germany (from 7.7 to 26.2%). VREFM was not observed in Spain, Sweden and Switzerland during the study interval. Daptomycin (DAP) was the most active compound against these organisms (see Table), followed by linezolid (LZD), and the activity of these compounds was not negatively affected by resistant (R) to oxacillin or vancomycin (VAN). All other compounds exhibited limited activity against VREFM. Among E. faecalis (956 strains), only 0.6% were VAN-R, while 77.8% of isolates were VAN-R, with 23.5% being VAN-S.

Conclusions: S. aureus and Enterococcus spp. represent important causes of BSI in European hospitals and the prevalence of MRSA and VREFM differs significantly among countries and has varied in many countries in recent years. DAP and LZD were the most active compounds tested against these MDR organisms as well as other GP pathogens isolated from episoles of BSI. Due to broad spectrum, potent bactericidal activity and approved indications, DAP represents a valuable treatment option for BSI caused by GP in European hospitals.

P1721 Antibiotic susceptibility pattern of Gram-positive cocci cultured from patients in three university hospitals in Tehran, Iran during 2001–2005

M. Emameini*, M. Aligholi, F. Jahalameli, S. Shahsavan, M. Taherkhali, S. Hossein, N. Jonaidi (Tehran, Iran, IR)

Objective: The antimicrobial susceptibility patterns of 1897 Gram-positive bacterial isolates were evaluated.

Methods: The minimum inhibitory concentration (MIC) of isolates which comprised Staphylococcus aureus (927 isolates), coagulase-negative staphylococci (CNS; 425 isolates), Enterococcus faecalis (320 isolates), Enterococcus faecium (157 isolates), and pneumococci (50 isolates) collected from 3 teaching hospitals in Tehran were determined by agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The presence of mecA gene was investigated in methicillin-resistant staphylococci by PCR method and vanA and vanB genes were targeted in enterococcal isolates by Multiplex PCR method.

Results: The resistance rate to methicillin among S. aureus and CNS isolates were 33% and 49%, respectively. All S. aureus isolates were susceptible to vancomycin. The lowest rate of resistance in all S. aureus isolates was found for rifampicin (<4%). The vancomycin resistance rate in enterococci isolates was 11% which was more frequent among E. faecium (19%) than E. faecalis (4%), all resistant isolates carrying vanA. High-level resistance to gentamicin and streptomycin, were detected in 47% and 87% of enterococcal isolates respectively.

Discussion: The rate of penicillin resistance in pneumococci was 3% and about 27% of isolates had reduced susceptibility to penicillin. The prevalence of erythromycin resistant among pneumococci was 58%. All pneumococcal isolates were susceptible to ceftriaxone, rifampicin and vancomycin.

P1722 Invasive pneumococcal infection in Scottish paediatric patients in the conjugate vaccine era

C. Lucas, C. Williams* (Glasgow, UK)

Introduction: Community acquired invasive infection in children is primarily mediated by capsulate bacteria, with infants at greatest risk due to poor responsiveness to carbohydrate antigens. The spectrum of infecting organisms has been dramatically altered by the Hib and MenC polysaccharide-protein conjugate vaccines and, following introduction of the heptavalent pneumococcal conjugate vaccine (PCV) into the routine UK immunisation schedule in 2006, this study was undertaken to examine pneumococcal strains associated with invasive disease in the local paediatric community.

Methods: Retrospective data, collected between January 1996 and December 2008, on invasive isolates of pneumococci cultured from paediatric patients in the West of Scotland, were examined. Serotype distribution and incidence of strains demonstrating reduced sensitivity to penicillin and erythromycin were calculated.

Results: Over the 13 year period, pneumococci were cultured from normally sterile sites in 216 patients. Of these, 127 patients were infected with potentially PCV preventable strains. Serotype 14a was the predominant strain, causing 25% of infections. MIC data, for penicillin and erythromycin, were available on 212 isolates. Seven strains with “intermediate” sensitivity to penicillin were cultured sporadically during the study period. No fully penicillin resistant isolates were grown. Forty erythromycin resistant strains were grown in total, 32 of which belonged to serotype 14a. With limited data available post introduction of PCV, no statistically significant change in infection rate or antibiotic sensitivity pattern is evident to date.

Conclusion: The majority of pneumococcal strains causing invasive infection in paediatric patients in the West of Scotland are included in PCV. A positive impact of this vaccine on infection rates and prevalence of antibiotic resistant strains remains to be demonstrated. Some 40% of infections in this study were caused by strains not included in PCV and the relative importance these requires to be carefully monitored over forthcoming years.
**P1723** Individual dose impact on resistance selection in the community: a mathematical model of *Streptococcus pneumoniae* dynamics and β-lactams


**Objectives:** *Streptococcus pneumoniae* is a major pathogen in the community. Controlling its resistance has become a public health priority. Increasing the dose, particularly of β-lactams, has been suggested to avoid failed treatments of infections caused by highly resistant bacteria.

**Methods:** To assess the impacts of antibiotic-exposure frequency and doses on resistance emergence, a mathematical model was constructed, combining *S. pneumoniae* pharamaco- and population-dynamic approaches in a community of individuals stratified according to their colonization and antibiotic-exposure status, and specifically including prescribed-dose heterogeneity in the population. Decolonisation could be either natural or induced by antibiotic exposure if the prescribed dose exceeded the carried strain's MIC. Simulations over a 50 years period were run to test the impact of dose-distribution and antibiotic-exposure frequency changes on community resistance patterns, and the accuracy of defined daily dose (DDD) as a predictor of resistance.

**Results:** After 50 years, nonsusceptible strain prevalence among carriers strongly reflected antibiotic exposure. Nonsusceptible strain prevalence increased with antibiotic-exposure frequency. The dose-distribution shift to higher doses had 2 paradoxical effects: while the prevalence of resistant strains dropped, these were comparatively more resistant as evidenced by larger MICs. Keeping the volume of antibiotic constant in exposure frequency changes on community resistance patterns, and the accuracy of defined daily dose (DDD) as a predictor of resistance.

**Conclusions:** Our results suggest that pneumococcal resistance pattern in the community is strongly linked to individual prescribed dose: recommendations to increase them should magnify nonsusceptible strain MIC in the community. Therefore, surveillance networks are encouraged to collect both daily antibiotic-exposure frequencies and individual prescribed doses.

**P1724** Invasive infections caused by drug-resistant *Streptococcus pneumoniae* at a Thai hospital

J. Vizuthrnnakul*, G. Suwanpimolkul, T. Chatsuwan, S. Nilgate, C. Suankratay (Bangkok, TH)

**Objective:** To determine the prevalence and clinical features of drug-resistant *Streptococcus pneumoniae* (DRSP) isolated from adult patients with invasive infections.

**Methods:** A retrospective study of all adult patients with *S. pneumoniae* bacteremia and meningitis who were hospitalised at King Chulalongkorn Memorial Hospital, Thailand, was carried out between 2004 and 2008.

**Results:** Of 65 pneumococcal isolates, there were 51 (78%) and 14 (22%) patients with bacteremia and meningitis. Of 51 patients with bacteremia, most common diagnosis was pneumonia (74.6%), followed by primary bacteremia (17.6%), skin and soft tissue infections (5.9%), and peritonitis (1.9%). Of these, there were 96%, 4%, and 0% of penicillin-susceptible *S. pneumoniae* (PSP), penicillin-intermediately resistant *S. pneumoniae* (PISP), and penicillin-resistant *S. pneumoniae* (PRSP), respectively. All isolates were susceptible to cefotaxime. Of 14 patients with meningitis, there were 43% and 57% of PSP and PRSP. In contrast to the observation in patients with bacteremia, cefotaxime-survivors, -intermediately resistant, and -resistant *S. pneumoniae* were noted in 72%, 14%, and 14%, respectively. All isolates from patients with bacteremia and meningitis were susceptible to vancomycin. Of 4 patients with cephalosporin-nonsusceptible *S. pneumoniae* meningitis, 3 had community-acquired meningitis and 1 developed meningitis after neurological operation. All of these patients survived with the treatment of ceftriaxone (4 g/day) alone. In our institute, the prevalence of penicillin- and cephalosporin-nonsusceptible *S. pneumoniae* in patients with meningitis has been markedly increasing from 22.2% to 57% and from 0% to 29%, respectively, during the periods of 1997–2003 and 2004–2008.

**Conclusions:** There has been an markedly increasing prevalence of penicillin- and cephalosporin-nonsusceptible *S. pneumoniae* in adult patients with meningitis in our institute. This emphasizes an urgent need to strengthen both appropriate use of antimicrobials and strict infection control measures to help reduce the transmission of DRSP.

**P1725** Emergence of antimicrobial resistance among Viridans group streptococci: a six-year surveillance in children attending day care centres


Resistance of Viridans Group Streptococci (VGS) is important to follow since they are a reservoir of resistant bacteria in oral cavities.

**Objectives:** to determine colonisation and resistance rates to antibiotics of VGS isolated from healthy children 3 months to 3 years old attending DCC in Nice (AM) in 2002, 2004, 2006 and Nord (N) in 2006 and 2008 (AM). NP aspirates were obtained from a random sample. Trends in resistance in pneumococcal strains (SP) and in VGS were compared. An intervention program to promote judicious antibiotic use started in AM in 2000, 2002 and a national campaign was launched in 2002.

**Methods:** Antibiotic susceptibility was determined by disk diffusion method and E-test on blood agar plates

**Results:** See the table.

<table>
<thead>
<tr>
<th></th>
<th>2002</th>
<th>2004</th>
<th>2006/06</th>
<th>2006/59</th>
<th>2008/06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
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<td>56.45</td>
<td>37.25</td>
<td>57.58</td>
<td>51.56</td>
</tr>
<tr>
<td>Amoxicillin</td>
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<td>29.41</td>
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<tr>
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<td>48.48</td>
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<td></td>
</tr>
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<td>Tetracycline</td>
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<td>30.65</td>
<td>27.45</td>
<td>42.42</td>
<td>42.19</td>
</tr>
<tr>
<td>Chloramphenicol</td>
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<td>5.88</td>
<td>24.24</td>
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</tr>
<tr>
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<td>48.48</td>
<td>54.69</td>
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<tr>
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<td>11.76</td>
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<tr>
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<td>1.96</td>
<td>0.00</td>
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</tr>
<tr>
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<td>56.45</td>
<td>66.67</td>
<td>51.52</td>
<td>100.00</td>
</tr>
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<td>6.56</td>
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<td>Moxifloxacin</td>
<td>8.20</td>
<td>7.84</td>
<td>12.12</td>
<td>49.21</td>
<td></td>
</tr>
</tbody>
</table>

A diminution of strains with decreased susceptibility to β-lactams was observed in 2006 only in AM and increase in resistance is observed in 2008.

The same evolution is observed for tetracycline, chloramphenicol and macrolides.

Resistance to fluoroquinolones is increasing although these antibiotics are not prescribed in this population of children.

**Conclusion:** Penicillin resistance of VGS (37% in AM and 57% in N) is equivalent to penicillin resistance of SP (34% in AM, 56% in N) in this children population in 2006 and increased in 2008 to 52% and 55% in N. Resistance of VGS is increased in 2006 and 2008 in VGS. For levofloxacin and moxifloxacin increased resistance was observed from 3% in 2004 to 35% in 2008, not easily comprehensive in this children population and which requires further evaluations.
Diverse populations of fluoroquinolone nonsusceptible group A streptococci recovered from colonisation and infections in Portugal (1999–2006)

(Caparica, Oeiras, Lisbon, PT, Barcelona, ES)

Objectives: To examine ciprofloxacin nonsusceptible (Cip-NS) Group A streptococci (GAS) isolated from clinical origins and asymptomatic colonisation, and to explore the associated clones and mechanisms of resistance.

Methods: A total of 1,541 GAS collected from oropharyngeal colonisation (n = 938), tonsillitis (n = 487), skin/soft tissue infections (n = 72) and invasive disease (n = 44) were studied. Susceptibility to ciprofloxacin was evaluated by disk diffusion and by microdilution methods. Point mutations in the parC-quinolone resistance determining region (QRDR) were identified by sequencing and by restriction of PCR amplicons with Hinfl and Lwel. Cip-NS isolates were characterised by emm-typing, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) to assign sequence types (ST).

Results: Eighty-one (5%) isolates were Cip-NS showing a MIC range between 2 and 8 mg/L. This rate was higher among skin/soft tissue infection isolates (15%, n = 11/72). All but three strains had ParC changes: S79F (n = 73), D83N (n = 4) and D83Y (n = 1). Seven ST or lineages, of diverse emm and PFGE types, were found among 81 Cip-NS isolates. A major lineage ST352/emm6;others/PFGE.AD;DX;DY;AM;DW (n = 73 isolates from different origins) showed the ParC-S79F change. Other Cip-NS strains were (all n = 1): ST52/emm28/PFGE.AC ParC-D83N, ST52/emm28/PFGE.BT ParC-D83Y, ST46/emm22/PFGE.B ParC-D83N, ST99/emm5/PFGE.BB ParC-D83N, ST-unassigned/emm5/PFGE.K ParC-D83N. The remaining 3 strains with no ParC changes were: ST36/emm12/PFGE.AP, ST39/emm4/PFGE.CH and ST39/emm89/PFGE.CZ. Lineages ST36 and ST-unassigned were associated with oropharyngeal colonisation isolates, ST39 and ST46 with tonsillitis and ST52 and ST99 with skin/soft tissue infections.

Conclusions: Nonsusceptibility to Cip among GAS in Portugal was similar to the one reported in European surveys. The majority of Cip-NS isolates belonged to emm6 (ST352) with S79F mutation however other different types emm28 (ST52), emm22 (ST46) or emm5 (ST99) found among colonisation and/or clinical isolates showed different ParC changes (D83N/Y). The absence of point mutations in the parC-QRDR region of emm4/89 (ST39) and emm12 (ST36) isolates suggests mechanism(s) of fluoroquinolone resistance other than point mutations in the parC-QRDR region (e.g., single parE mutations and/or efflux pumps).

Trends in invasive group A streptococci in adult patients in Barcelona

C. Ardanuy*, D. Rolo, A. Domenech, F. Tubau, J. Ayats, R. Martin, J. Liñares, C. Ardanuy* (L’Hospitalitat de Llobregat, ES)

Objective: The aim of this study was to analyze the evolution of phenotypes, genotypes and macrolide resistance genes of Streptococcus pyogenes (GAS) isolated from invasive and non-invasive disease among adults patients from 1998 to 2007.

Methods: A total of 445 GAS strains were isolated in our hospital during the study period. Antibiotic susceptibility was studied by microdilution and macrolide resistance phenotypes by disk diffusion. Molecular typing was performed by PFGE (Smal) and emm-typing, and selected strains were studied by MLST. Macrolide resistant genes and those of Tn916-family of transposons were detected by PCR.

Results: During the study period 95 (21%) erythromycin resistant isolates were detected. Of them, 50 occurred in men (53%). The medium age was 49 (SD 19, range 18–90). The 59% of them were isolated from soft-tissue infections, 15% from respiratory tract specimens and 6% from pharyngitis. By two year period the rates of erythromycin-resistant (EryR) were: 18.42% (1998–1999), 15.58% (2000–2001), 45.6% (2002–2003), 25.49% (2004–2005) and 9.84% (2006–2007). Thirty-six (37.9%) strains had M phenotype and 59 (62.1%) had MLSB phenotype (39 cMLSb and 20 IMLSb). The proportion of M decreased throughout the study period (from 10/14 in 1998–99 to 4/12 in 2006–2007, p = 0.05). All 26 M phenotype GAS studied had mefA gene. Of 21cMLSb studied 20 had ermB and 1 had ermTR genes. Five IMLSb GAS had ermTR and 8 had ermB. No tetracycline resistance nor tetM gene was detected among M phenotype GAS. All GAS harbouring ermB gene had tetM gene and the majority of them (75%) were related to Tn916-family of transposons (Tn6002 and Tn1545). Three GAS with ermTR gene were resistant to tetracycline related to tetM gene probably harbouring Tn916 transposon. The predominant genotypes were emm11-ST403 (12%), emm4-ST39 (7%) and emm25-ST350 (n = 6%).

Conclusion: Macrolide resistance rates among GAS isolated from adults fluctuates throughout the study period. The peak observed in 2002–2003 period was associated with an outbreak due to emm25-ST350 among injection drug users. The increased rates of MLSB phenotype were associated with the spread of Tn916-family of transposons harbouring ermB and tetM genes.

Oropharyngeal colonisation by group A streptococci in Portugal: an eight-year surveillance study (2000–2007)


Objectives: To evaluate trends of oropharyngeal colonisation (OC) by Group A streptococci (GAS) and to assess the clonal structure of sporadic and persistent strains.

Methods: During 16 periods in 2000–07, oropharyngeal samples were taken from different populations: 6965 from children (0–6yrs) in day-care centres (DCC), 2337 from school-aged children (7–16yrs)
and 1276 from adults (1169 school staff and 107 family members). Bacterial identification was carried out by standard methods. Resistance to erythromycin (E) and clindamycin (Da) was evaluated by disk diffusion and minimal inhibitory concentrations using E-tests. Clones of all resistant and a subset of susceptible isolates were defined by pulsed-field gel electrophoresis (PFGE) and further characterised by serotyping for T antigen (T-typing), and by sequencing for assignment of emm-types and multilocus sequence types (ST).

**Results:** A total of 1026 GAS were isolated. OC in younger children was higher (11.6%) than among older than 7 yrs (7.8%), and in adults was higher among family members (8.4%) than among school staff (2.6%). OC rates varied with DCC (min. 0%; max. 49%). Higher Results:

Field genetic electrophoresis (PFGE) and further characterised by serotyping all resistant and a subset of susceptible isolates were defined by pulsed-field gel electrophoresis (PFGE) and further characterised by serotyping.

- **Methods:** A 20-month study of CDI was conducted at the University Hospital in a Chinese university hospital.

**Conclusions:** Toxigenic isolates were more frequently detected in patients with chronic obstructive pulmonary disease, cystic fibrosis and bronchiectasis. The highest number of cases (n=4) was identified in 2008. The median age was 54.3 years old and 60% were male. The most frequent underlying diseases in patients with infection were cancer in 20%, solid organ transplantation 20%, HIV infection 12% and diabetes mellitus 12%.

**Results:** Of 136 isolates, 104 were from the first episode of CDI, 13 cases were from re-infections, and 19 were toxin-negative. The 107 nonrepeated and toxigenic isolates, 76 were A patients presented immunosuppression as a predisposing factor. TMS presented a good in vitro activity, but it was frequently switched to another antibiotic due to intolerance or lack of efficacy. Susceptibility tests showed that amikacin plus imipenem cover Nocardia spp. isolated in our institution. This combination could be considered the treatment of choice for nocardiosis. Sensitivity to imipenem cannot be extrapolated to other carbapenems.

**P1731** Characterisation of clinical *Clostridium difficile* isolates by rep-PCR, PFGE and PCR ribotyping


**Objectives:** *Clostridium difficile* is a major cause of nosocomial infections. In recent years, a highly virulent strain (NAP1/ribotype 027) that causes more serious disease and increased mortality has emerged in both Europe and North America. Tracking the source and spread of these infections through strain-typing will help to contain hospital associated infections. Several molecular typing methods are used as tools to strain-type *C. difficile*, including PFGE and PCR ribotyping. An automated repetitive sequence-based PCR system, the DiversiLab System, has also been used in strain typing of *C. difficile*. This study compares rep-PCR, PCR ribotyping and PFGE methods for strain typing.

**Methods:** A total of 84 *C. difficile* consisting of clinical isolates from a healthcare facility in the UK (17 isolates) and a healthcare facility in the US (67 isolates) were previously characterised by PCR ribotyping. The sample set consisted of 25 different PCR ribotypes including multiple ribotype 027. Each isolate was cultured and genomic DNA was extracted using the UltraClean™ Microbial DNA Isolation Kit. For rep-PCR, DNA was amplified using the *Clostridium* Kit for DNA Fingerpinting. The amplified product fragments were separated using microfluidics lab-on-a-chip technology and analyzed using web-based data analysis software. PFGE was carried out using the CHEF-DRTMII (Bio-Rad Laboratories) electrophoresis system following a previously described method.

**Results:** Rep-PCR and PCR ribotyping fingerprints were generated for every sample. PFGE fingerprints were generated for over 95% of isolates. Rep-PCR, PFGE and PCR ribotyping clustering showed a high concordance; however, rep-PCR and PFGE showed a higher level of discrimination by divisions within some ribotype clusters. Additionally, rep-PCR and PFGE provided multiple fingerprints for ribotype 027.

**Conclusions:** Rep-PCR and PCR ribotyping had a more rapid turnaround time and were more robust because DNA degradation was less of a concern as compared to PFGE. However, both PFGE and rep-PCR provided a higher level of discrimination than PCR ribotyping including for isolates of ribotype 027.
An epidemiological study of detection rates of European Surveillance of Antimicrobial Consumption

This study suggests that enlargement of the infection control team was increased and an isolation ward was increased. Resistance to moxifloxacin, ciprofloxacin, levofloxacin, erythromycin, clindamycin, tetracycline, rifampin, fusidic acid and meropenem was found in 43.9%, 100%, 78.5%, 72.6%, 79.2%, 40.2%, 20.6%, 17.6% and 0.9% of the isolates, respectively. Thirty-two strains exhibited multiresistance to erythromycin/cloxacin, moxifloxacin and tetracycline. The prevalence of resistance genes in the isolates was as follows: ermB, 23; ermFS, 0; tetM, 32; gyrA mutation, 22; gyrB mutation, 2; gyrA and gyrB mutation, 8.

Conclusions: One special clone (SH II) dominated and ribotype 027 was not found. The prevalence of combined drug resistance is high and the growing load of resistance mechanisms needs more investigations. Further epidemiological surveillance of CDI is required to detect clustering of cases and to monitor the emergence of specific highly virulent clones in China.

An epidemiological study of detection rates of Clostridium difficile toxins A and B at a hospital in King’s Lynn, England from 2000 to 2008

C. Micallef*, G. Rogerson, L. Liebowitz (King’s Lynn, UK)

Introduction: Clostridium difficile associated diarrhoea (CDAD) is an important cause of hospital-acquired infection. It has been estimated that in the United Kingdom, this accounts for an additional cost of £400,000/100 patients [1]. Predisposing factors include: antibiotic therapy, age 65 years or more, chemotherapy, proton pump inhibitors, and increased hospital stay [2,3]. Additional risk factors include gastric acid suppression medications [3]. C. difficile produces a number of toxins, notably, Toxin A (TcdA), an enterotoxin and Toxin B (TcdB), a cytotoxin4.

Objectives:
- Exploring the epidemiological pattern of toxin-producing C. difficile at The QEH during an eight-year period.
- Investigating impact of infection control interventions.

Methodology: The QEH is a district general hospital in the East of England, with 480 beds, providing a service to 280,000 people. Stool samples which were submitted for testing to the Microbiology Department from 2000 to 2008 were screened for C. difficile toxins A and B. Use of cephalosporins and quinolones was discouraged from July 2005. The infection control team was increased and an isolation ward introduced in 2008.

Results: Highest rates occurred in 2005 with a total of 546 cases. From 2005 to 2008 there was a progressive decrease in rates of C. difficile toxin detection to 129.

Conclusions: This study suggests that enlargement of the infection control team, introduction of isolation ward and antibiotic prescribing guidelines, minimising the use of cephalosporins and quinolones has had a direct impact on the C. difficile infection rates at The QEH.

Reference(s)

Antifungal therapy

European Surveillance of Antimicrobial Consumption (ESAC): outpatient antymycotic use in Europe

N. Adriaensen*, S. Coenen, A. Muller, V. Vanerkhochten, E. Hendrickx, H. Goossens on behalf of the ESAC Project Group

Objectives: To assess the total outpatient systemic antymycotic use in Europe and to identify the antymycotic substances most commonly used.

Methods: The European Surveillance of Antimicrobial Consumption (ESAC; www.esac.ua.ac.be) project, now funded by the European Center for Disease Control and Prevention (ECDC; agreement number 2007/001), continues to collect data on antimycotic consumption for all Member States, candidate countries and European Free Trade Association-European Economic Area countries using the anatomic chemical therapeutic (ATC) classification and the defined daily dose (DDD) measurement unit. For 2007, data on outpatient use of all antymycotics for systemic use (ATC J02 and D01B), aggregated at the level of the active substance, was collected and use was expressed in DDD (WHO ATC/DDD, version 2007) per 1000 inhabitants per day (DID). Only countries for which data on both J02 and D01B use was available were included in the analysis.

Results: Total outpatient antymycotic use in 2007 in 11 European countries (data for Estonia include hospital use) varied by a factor of 11.65 between the country with the highest (3.03 DDD in Belgium) and the country with the lowest (0.26 DDD in Slovakia) use. Terbinafine, itraconazole, and fluconazole were the most prescribed substances, and represented more than 96% of the total outpatient antymycotic use in all countries except for Estonia, Slovakia and Latvia (83.8%, 78.5% and 74.6%, respectively). Terbinafine use represented more than 50% of the total outpatient antymycotic use in 8 out of 11 countries (not in Latvia, Hungary and Italy).

Figure: Total outpatient systemic antymycotic use in 11 European countries in 2007.
Conclusion: Our study demonstrates a variation of outpatient systemic antifungal use in Europe as striking as that of outpatient systemic antibiotic use. The ESAC data facilitate auditing of antifungal prescribing and evaluation of the implementation of guidelines and public health policies to promote its judicious use.

P1735 Management of candiduria: an interview schedule
V. Akan-Oguz*, N. Yavuz, M. Acici, G. Mermut, H. Pail iukcu, S. Sacar, S. Sayin-Kulh, B. Cetin, B. Ertugrul on behalf of the West Anatolian Fungal Infection Study Group

Objectives: Management of candiduria remains controversial, mainly due to uncertainties of clinicians how to diagnose and when to start treatment. In this study we aimed to investigate diagnostic and therapeutic approach of different specialists for candiduria.

Methods: An interview schedule composed of 10 questions on candiduria was applied interactively to 393 clinicians during three months in six different tertiary care hospitals. We separated the questions into two parts. First part was about demographic features, following up of Candida guidelines and the advice of participants for patients who have Candida growth in the first urine culture. Second part consisted of six questions about diagnostic and therapeutic approach. These questions were not asked to participants who did not suggest second urine culture after first positive one. We compared the answers of infectious diseases (ID) specialists with the others (internist, surgeon, and intensivist). The data of each participant were evaluated using the Statistical package for Social Sciences version 11. For statistical analyses chi-square test was used.

Results: Of 393 participants, 88 (22.4%) were ID and 305 (87.6%) were other specialists (199 internists, 79 surgeons, 27 intensivists) specialists. Of the participants, 215 (54.7%) were female, 178 (45.3%) were male and mean age was 34.27±8.4 (24–63). The number of participants diagnosing candiduria more than 20 times in a year was 76 (19.4%). The details of interview are presented in the table. The difference between the compliance of ID and other specialist to the guidelines or literature about diagnosis and starting therapy for candiduria was statistically significant. First choice of therapy was amphotericin B for neutropenic patients and fluconazole for nonneutropenic patients.

Conclusion: The ID specialists displayed a more proactive and consistent approach to Candiduria treatment than the other specialists. This approach was especially observed in the high-risk patient group and also in utilising the second urinary culture. Accordingly, the above mentioned results, point to a room for improvement in the management of candiduria, aiming for a consensus.

<table>
<thead>
<tr>
<th>Choice of therapy for patients without neutropenia</th>
<th>ID specialists</th>
<th>Other specialists</th>
<th>Statistic</th>
</tr>
</thead>
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<tr>
<td>Fluconazole</td>
<td>29 (39.2)</td>
<td>39 (29.7)</td>
<td></td>
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<tr>
<td>Other antifungal agent</td>
<td>48 (68.0)</td>
<td>91 (70.3)</td>
<td></td>
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<tr>
<td>Outcome of candiduria</td>
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<tr>
<td>Spreading of funga into blood cultures</td>
<td>57 (78.3)</td>
<td>104 (79.7)</td>
<td>0.116</td>
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<tr>
<td>Other antifungal agents</td>
<td>2 (2.7)</td>
<td>14 (10.8)</td>
<td></td>
</tr>
</tbody>
</table>

P1736 Empirical antifungal therapy in selected patients with persistent febrile neutropenia
A. Martín-Peña*, M. Aguilar-Guisado, J.M. Cisneros, E. Cordero, I. Espigado, R. Parody, J. Puchín for the Spanish Network for Research in Infectious Disease (REIPI)

Objectives: The aim of this study is to analyze the incidence and related mortality of invasive fungal infection (IFI) in patients with persistent febrile neutropenia, using empiric antifungal therapy (EAT) only in selected patients by risk factors and clinical criteria.

Methods: Prospective observational study including every persistent febrile neutropenia episodes in patients with haematological malignancies or stem cell transplantation (SCT) recipients admitted in the Haematology Service from October 2007 to November 2008. A diagnostic and therapeutic protocol based on clinical criteria and on the infection risk factors was applied in every episode in order to select patients for EAT indication. Comparative analysis of incidence of proven or probable IFI and IFI-related mortality in patients with persistent febrile neutropenia according to the indication or not of EAT.

Results: Fifty three episodes PFN in forty six patients were included. The 56.5% were male and the median age in years was 51 (15–71). The most frequent haematological malignancies were acute leukaemia (36.9%) and lymphoma (26.1%). Eighteen patients were SCT recipients, 50% allogeneic. The 21.7% were IFI-high risk patients. The mean of duration of neutropenia and fever were 14 days (range: 6–63) and 11 days (range: 4–33) respectively. A diagnostic of proven or probable infection was established in 79.2% of the cases. The most frequent clinical syndromes were: respiratory (35.8%) and non focused fever (35.8%).

Choice of therapy was amphotericin B for neutropenic patients and fluconazole for nonneutropenic patients.

Conclusion: These data suggest that EAT in selected patients used in the basis of clinical criteria and risk factors, may be effective and safe in the management of PFN and avoid overtreatment.

P1737 Outcomes associated with antifungal treatment and fluconazole treatment failure for candidaemia or invasive candidiasis in patients admitted into intensive care units

Objectives: To compare the resource utilisation and outcomes within patients with candidaemia or invasive candidiasis (IC) admitted in ICU.

Methods: We used data from 5 large Spanish hospitals by retrospectively clinical charts reviews. Patients who started from January 07 to April 08 on the following criteria: primary or secondary diagnosis using ICD-9 codes for candidaemia or IC receiving any IV antifungal drug (AF), age > 18, and admitted in ICU. Patients were classified in two groups: those who started with fluconazole (FLC) and those who started with any other licensed AF therapy (OLAT). FLC success was considered when those who started with fluconazole (FLC) and those who started with any other licensed AF therapy (OLAT). FLC success was considered when those who started with the start of AF until discharge from ICU or death.

Results: A total of 43-patients met inclusion criteria, 36 of whom started on IV FLC, and 7 on OLAT (4 caspofungin, 1 AmB-L, 1 AmB-LC, 1 voriconazole). Mean age (SD) was 61.8 (14.7) years in the FLC arm vs. 46.9 (16.5) in the OLAT (p > 0.05); 63.9% men vs. 57.1% (p < 0.05), respectively. Sixty-four percent of patients had an average of one underlying disease in the FLC arm, while 57.1% in the OLAT (p < 0.05), where the severity disease was “rapidly fatal” 58.3% vs.
The effect of an antimycotic restriction programme
Liposomal amphotericin B in intensive care unit patients

Mortality rates due to the fungal infection were higher in the OLAT arm. Caspofungin was administered as predominant antifungal treatment in the majority of patients (29 out of 107 patients). After implementation of the antifungal restriction policy, the overall incidence of candidaemia in the 2 abdominal surgical departments and intensive care units decreased significantly from 17 cases of 203 bacteraemia cases in 2006 to 5 cases of 205 bacteraemias in 2008 (p=0.01). The amount and nature of surgical procedures performed and the number of discharges did not differ considerably in these 2 years and no other changes in routine protocols were considered to have affected the outcome. The most frequently isolated organism was Candida albicans (13 cases, 57%), followed by C. glabrata (7 cases, 30%), 2 isolates were C. krusei (9%) and 1 C. tropicalis (4%). One case was a mixed infection with C. glabrata and C. krusei. All of the 5 cases in 2008 (4 C. albicans and 1 C. tropicalis) were fluconazole-susceptible. The clinical picture was predominated, as expected, by 14 cases (64%) having an intraabdominal focus. Four cases (18%) were intravenous catheter infections and in the remaining 4 cases (18%) there were more than 1 possible focus. Thirty-day mortality was 55% for all 22 cases taken together.

Conclusion: The incidence of candidaemia decreased significantly from 2006 to 2008 in abdominal surgery patients in 2 Copenhagen hospitals. The addition of fluconazole to the prophylactic and early presumptive antibiotic treatment recommendations in our hospitals in Copenhagen for patients with documented or suspected gastrointestinal tract perforation and necrotising pancreatitis. The aim of this study was to examine the effectiveness of this intervention.

Methods: All positive blood culture results in our department of clinical microbiology serving 6 hospitals are registered prospectively and supplemented by clinical information. This is part of a collaborative network between 3 departments of clinical microbiology and a seminational database of bacteraemia. The rates and clinical characteristics of candidaemia and bacteraemia in the abdominal surgical departments and surgical intensive care units of the 2 major hospitals were compared in the years 2006 and 2008.

Results: The overall incidence of candidaemia in the 2 abdominal surgical departments and intensive care units decreased significantly from 17 cases of 203 bacteraemia cases in 2006 to 5 cases of 205 bacteraemias in 2008 (p=0.01).

The amount and nature of surgical procedures performed and the number of discharges did not differ considerably in these 2 years and no other changes in routine protocols were considered to have affected the outcome. The most frequently isolated organism was Candida albicans (13 cases, 57%), followed by C. glabrata (7 cases, 30%), 2 isolates were C. krusei (9%) and 1 C. tropicalis (4%). One case was a mixed infection with C. glabrata and C. krusei. All of the 5 cases in 2008 (4 C. albicans and 1 C. tropicalis) were fluconazole-susceptible. The clinical picture was predominated, as expected, by 14 cases (64%) having an intraabdominal focus. Four cases (18%) were intravenous catheter infections and in the remaining 4 cases (18%) there were more than 1 possible focus. Thirty-day mortality was 55% for all 22 cases taken together.

Conclusion: The incidence of candidaemia decreased significantly from 2006 to 2008 in abdominal surgery patients in 2 Copenhagen hospitals. The addition of fluconazole to the prophylactic and early presumptive antibiotic regimen is the most likely reason for that change. The study’s main limitations are the small case numbers and the descriptive nature of the study design.

Objective: Efficacy and safety of Liposomal Amphotericin B (L-AmB) in intensive care unit patients previously treated with fluconazole. Methods: Retrospective, multicentre study of patients admitted to ICUs and treated with L-AMB as second line treatment after fluconazole. Invasive fungal infections (IFIs) were classified as proven, probable or possible.

Results: 41 patients were included, 60% were male and median age was 62 years. Mean length in ICU was 31.8 (SD:22.6) days and mortality rate in ICU 59%. Mean APACHE II score was 20.7 (SD:7.8) and at admission 34% of the patients had severe sepsis or septic shock. Most common pathologies were surgery (40%) and medical pathology (39%). Invasive fungal infections were proven, probable and possible in 42%, 19% and 32%, of patients, respectively, and not classified in 7%. Most common fungi identified (it was possible several species) were C. glabrata (56%)
Continuous infusion of amphotericin B deoxycholate in the treatment of cryptococcal meningoencephalitis: analysis of safety and fungicidal activity


Objective: Cryptococcosis is a deep mycosis commonly seen in immunocompromised hosts. The mainstay of treatment remains amphotericin B deoxycholate, which is associated with nephrotoxicity and other adverse events. New lipid formulations provide a good solution to toxicity concerns. Reduction in nephrotoxicity and side effects can be explained by slower drug distribution in tissues. However, costs of these formulations are prohibitive. In this scenario, continuous infusion of amphotericin B deoxycholate for the treatment of serious fungal infections like cryptococcosis could reveal a promising strategy. Our objective in this study is to evaluate safety, clinical and microbiological efficacy of continuous infusion of amphotericin B deoxycholate in treatment of cryptococcal meningoencephalitis.

Methods: Non-comparative clinical trial, including patients with disseminated cryptococcosis. All patients received continuous infusion of amphotericin B deoxycholate (0.7 mg/kg daily) and oral flucytosine (25 mg/kg four times a day), during induction phase of 14 days. We measured fungicidal activity using serial quantitative cultures of (25mg/kg four times a day), during induction phase of 14 days.

Results: Analysis of data on 6 patients has shown that all patients presented a progressive reduction in CFU cryptococcal colony-forming units (CFU), and LCR was sterile at 2 weeks of treatment. An exponential reduction of CFU counts was observed (figure 1). Although two patients developed severe hypokalaemia, glomerular renal function was well preserved in all patients with creatinine serum levels below 1.5 mg/dL at end of 14 days of antifungal therapy. We observed the occurrence of anaemia (decrease of haemoglobin at least of 3 g/dL) in 3 patients.

Conclusions: The preliminary data presented here are indicating that continuous infusion of amphotericin B seems to be safe and well tolerated, despite the development of anaemia and hypokalaemia in some patients. Mycological efficacy was comparable as standard of treatment, with adequate reduction rate of CFU and sterilisation of CSF. These results could suggest that continuous infusion reduces nephrotoxicity while keeping fungicidal activity. Larger and comparative trials, against new lipid formulations and even novel antifungal agents, are necessary to further evaluate this treatment regimen.

Efficacy and safety of liposomal amphotericin B in intensive care unit patients with confirmed invasive fungal infection: a retrospective, multi-centre study

F. Alvarez-Lerma, J. Diaz, F. Martiscal, M. Nieto, F. Assin* on behalf of the Study Group for Liposomal Amphotericin B

Objective: Invasive fungal infections (IFI) are a frequent life-threatening complication in high risk patients hospitalised in ICUs. The objective of this study was to analyse the efficacy and safety of liposomal amphotericin B (L-AMB) administered to ICU patients with confirmed IFI.

Methods: Retrospective, multicentre and observational study of patients admitted to ICUs with confirmed fungal infection and treated with L-AMB.

Results: Seventy-eight patients were included, 57.1% were male and median age was 56 years. Mean time in ICU was 46.4 (SD: 50.4) days and mortality rate in ICU was 42.7%. Mean APACHE II score was 21.3 (SD: 8.0) and severe sepsis or septic shock was 65.4%. Most common pathogens were medical (4.3%) and surgery pathalogy (42.3%). Most common proven IFIs were Candida albicans (56.4%), Candida glabrata (14.1%), Candida parapsilosis (11.5%) and Aspergillus spp (7.7%). Mean duration of treatment was 16.5 days and mean dose was 3.7 mg/kg/day. Previous antifungal treatment was administered to 56.4% of patients, mainly fluconazole (29.5%) and Caspofungin (21.8%). Most common reasons L-AMB was initiated were: non-stable disease (48.7%), infection localisation (38.5%) and L-AMB spectrum (34.6%). Satisfactory clinical response (Complete and partial response) was achieved in 68% (95% CI: 57.6, 78.3) of the patients and microbiological response (negative culture) in 61.5% (95% CI: 50.7, 72.3) of the patients. Within evaluable patients these results were: 72.6% (95% CI: 62.4, 82.8) and 76.2 (95% CI: 65.7, 86.7). 34 related AEs were reported, but only 2 were reported as serious: a case of hypokalaemia and a case of renal failure requiring a change in the antifungal treatment. There was no change in the global mean creatininc value at the end of treatment in the patients treated with L-AMB, despite the fact that 52.6% were receiving nephrotoxic drugs concomitantly.

Conclusion: L-AMB was used in critically ill patients (haemodynamically unstable) with confirmed IFI, including a high proportion who had received previous therapy for IFI. Satisfactory clinical and microbiological response in evaluable patients was high. L-AMB was...
well tolerated with little alteration of renal function even in patients taking concomitant nephrotoxic drugs. L-Amb can be considered an effective and safe option in confirmed IFIs in critically patients.

**P1743** Miconafungin versus liposomal amphotericin B for the treatment of serious Candida infections in intensive care unit and non-ICU patients: results of post-hoc analyses


**Objective:** Assess efficacy and safety of miconafungin (MICA) versus liposomal amphotericin B (L-AmB) for the treatment of invasive candidiasis (IC) or candidemia in ICU and non-ICU patients and identify factors associated with outcomes.

**Method:** Post-hoc analyses of data from a phase 3 non-inferiority trial of MICA (100 mg/day for patients >40 kg; 2 mg/kg/day for patients ≤40 kg) vs L-AmB (3 mg/kg/day). Subgroups were defined according to the type of ward on the first day of treatment: ICU or non-ICU. Multivariate regression analyses were performed to identify factors associated with treatment success at end of therapy and all-cause mortality at days 8 and 30 post-treatment initiation. Analytical model, ICU status no longer emerged as a significant associated variable but the association between APACHE II score and treatment outcome remained. Further analyses indicated that the likelihood of mortality at day 8 and day 30 was lower for patients with lower APACHE II scores. Renal function was significantly better in MICA versus L-AmB patients: a difference (L-AmB-MICA) in mean peak change in estimated IIscores. Renal function was significantly better in MICA versus L-AmB patients: a difference (L-AmB-MICA) in mean peak change in estimated IIscores.

**Results:** In non-ICU patients, treatment success was significantly higher for MICA vs L-AmB (85% [n=108/127] vs 72.1% [n=98/136]; P=0.0113). However, for ICU patients, treatment success rates for MICA vs L-AmB were similar (62.5% [n=75/120] vs 66.4% [n=73/110]; not significant). Overall, treatment success was significantly lower in ICU patients compared with non-ICU patients (64.3% [n=148/230] vs 78.3% [n=290/263]; P=0.0006). Furthermore, multivariate regression analysis revealed a lower likelihood of treatment success for: ICU vs non-ICU patients; patients with persistent neutropenia during therapy; and patients with high versus low APACHE II scores. However, when interactions between potential explanatory factors were included in the analysis model, ICU status no longer emerged as a significant associated variable but the association between APACHE II score and treatment outcome remained. Further analyses indicated that the likelihood of mortality at day 8 and day 30 was lower for patients with lower APACHE II scores. Renal function was significantly better in MICA versus L-AmB patients: a difference (L-AmB-MICA) in mean peak change in estimated glomerular filtration rate (mL per min per 1.73 m2) of ~18.2 (P<0.0001) and ~17.7 (P=0.0124) in non-ICU and ICU patients, respectively.

**Conclusion:** Overall, ICU patients had lower treatment success rates than non-ICU patients for both L-AmB and MICA. When controlling for confounding factors, multivariable analysis revealed that APACHE II score remained the only potential explanatory factor associated with treatment success, mortality at day 8, and mortality at day 30. Further characterisation of this finding and its implications is required.

**P1744** High rate of breakthrough invasive aspergillosis among patients with persistent fever and neutropenia receiving empirical caspofungin therapy


**Objectives:** A number of agents are now available for empirical antifungal treatment (EATF) of patients with persistent fever and neutropenia. We wished to study the antifungal drugs used in our institution and their efficacy to prevent breakthrough invasive fungal infections.

**Methods:** A prospective study was carried out from November 2005 to February 2006 by reviewing the medical records of all consecutive patients admitted in haematology, oncology, intensive care and infectious diseases wards who received EATF. Baseline patients characteristics at the time of EATF initiation were recorded as well as the type, dose and duration of the antifungal agents used. Breakthrough invasive fungal infections were documented according to the EORTC/MSG definition. Patients were followed until they were discharged from the hospital or died.

**Results:** Sixty-three episodes of persistent fever with neutropenia requiring EATF were recorded among 56 patients. All patients received high dose chemotherapy for other acute myeloid leukaemia (52%), acute lymphoid leukaemia (14%), lymphoma (13%) or other haematologic conditions (21%). Fifteen (27%) and 5 (9%) patients were allogeneic and autologous haematopoetic stem cell transplant recipients, respectively. Caspofungin was prescribed initially for 40 episodes (63.5%), amphotericin B (Amb) deoxycholate and liposomal Amb for 12 (19%) and 11 (17.5%) episodes, respectively. Six patients were switched from liposomal Amb to caspofungin because of adverse events. Median duration of therapy was 9 days (1–42) for caspofungin, 9 days (1–31) for Amb and 4.5 days (1–22) for liposomal Amb. During follow-up, 7 patients (11.1%) were diagnosed with invasive aspergillosis (IA), after a median of 7 days (3–19) of EATF. IA were probable in 4 cases and possible in 3. IA occurred in 6/46 (13%) of caspofungin recipients and in 1/23 (4.3%) of Amb (deoxycholate and liposomal) recipients (OR: 3.3).

**Conclusion:** We documented in our study a high rate of breakthrough invasive aspergillosis among high risk patients receiving preferentially caspofungin for EATF. The association between the use of caspofungin and the risk of invasive aspergillosis requires further evaluation.

**P1745** Cost-effectiveness of anidulafungin in confirmed candidaemia and other invasive Candida infections in Spain

S. Grau, M. Garcia-Vargas, B. Martí*, N. Mir (Barcelona, Alcobendas, Madrid, ES)

**Background:** Candidaemia and other invasive Candida infections are economically costly and can cause patient death. Anidulafungin, a newly licensed candin, has shown to be effective in treating candidaemia. The aim of the study is to evaluate the cost-effectiveness of anidulafungin compared with current standard of care, fluconazole, for the treatment of invasive candidiasis and candidaemia in Spain.

**Methods:** A decision tree model from the hospital perspective was constructed to examine the cost-effectiveness of anidulafungin compared with fluconazole in the treatment of confirmed candidaemia. Treatment success, patient treatment patterns, and patient survival were based on the results from a randomised, double-blind multicentre trial (Reboli AC, et al. N Engl J Med 2007). Only in-hospital (€2007) direct costs per patient were considered. Costs were obtained from a Spanish national database. Renal toxicity probabilities and costs were extracted from the published literature. The incremental cost per successfully treated patient was calculated. One-way sensitivity analyses were performed to test the robustness of the model.

**Results:** The percentage of successfully treated patients at the end of all therapy was higher for patients treated with anidulafungin than with fluconazole (74% vs. 57%). Treating with anidulafungin resulted in a higher antifungal drug costs (5,780€ vs. 2,082€); however, overall costs are lower for treatment with anidulafungin than for treatment with fluconazole (37,240€ vs. 37,327€) due to an offset in other medical costs. Univariate sensitivity analyses showed that anidulafungin remained the most cost-effective option.

**Conclusions:** Anidulafungin has demonstrated to improve the clinical efficacy versus standard of care in treating confirmed candidaemia. Despite an increase in drug costs, treating confirmed candidaemia with anidulafungin is a cost-effective strategy.

**P1746** Economic evaluation of anidulafungin (Eraxis®) versus intravenous fluconazole in the treatment of hospital inpatients diagnosed with candidaemia and other forms of invasive candidiasis


**Objective:** In clinical trial anidulafungin (ANI) achieved higher global response rates at the end of therapy (EOT) and 2 weeks after EOT.
compared with intravenous fluconazole (FLU) in the treatment of candidaemia/invasive candidiasis (CIC; P = 0.01). We assessed the economic impact of this therapy in hospital inpatients with CIC.

**Methods:** Clinical and resource use data for 234 hospital inpatients with CIC who received ≥1 dose of study drug from North American sites participating in a Phase 3, randomised clinical trial comparing ANI with FLU were retrospectively analyzed through chart review methodology. Charts were available for 159 patients. To estimate missing intensive care unit (ICU) length of stay data for the remaining 75 patients, two approaches were evaluated: a blinded adjudication process conducted by 4 clinicians; and a regression imputation method. Treatment efficacy and CIC-related cost to a US hospital payer were evaluated over a 13-week period from the initiation of treatment. One-way sensitivity analyses were conducted to assess the robustness of the results.

**Results:** Patients treated with ANI achieved a higher global response rate by EOT (57.5% vs. FLU; 39.5%; P = 0.02). Over a 13-week period total hospital days and ICU days were similar for ANI and FLU (total: 28.6 vs. 26.0, P = 0.20; ICU-adjudication: 7.8 vs. 9.1, P = 0.77; and ICU-regression 9.8 vs.10.7, P = 0.72). Compared to FLU, patients treated with ANI appeared to spend more days alive out of the hospital (47.2 vs. 43.6, P = 0.65), when assessed at 13 weeks. After adjusting for baseline APACHE II and other factors, the costs were similar in the two treatment groups (total cost-adjudication: $44,781 vs. $42,558, P = 0.70; total cost-regression: $47,658 vs. $44,977, P = 0.66).

**Conclusions:** In hospital inpatients with CIC, treatment with ANI was associated with superior clinical response with no increase in cost compared to treatment with IV FLU.

**P1747** Antifungal therapy with voriconazole improves outcome in patients with invasive pulmonary aspergillosis and chronic obstructive pulmonary disease

J. Guinea, M. Torres-Narbona, P. Gijón, J. de Miguel, P. Muñoz, T. Peláez, E. Bouza (Madrid, ES)

**Objectives:** Invasive pulmonary aspergillus (IPA) in patients with chronic obstructive pulmonary disease (COPD) is an emerging fungal infection with a high mortality rate, despite antifungal therapy. However, first-line antifungal therapy for IPA in this population has not been properly established. We determined risk factors that predict poor outcome in patients with COPD and IPA, with emphasis on the role of antifungal therapy.

**Methods:** From November 1999 to March 2008, we evaluated 57 cases of probable IPA in patients with COPD. We reviewed their charts for clinical features, bacterial co-infections, analytical data, previous use of corticosteroids/antibacterial agents before the isolation of Aspergillus, antifungal treatment, and outcome.

**Results:** The 57 patients with IPA showed an overall mortality of 71.9%. We detected 4 independent variables able to predict poor outcome (P < 0.05) (OR, 95% CI): “chronic heart failure” (38.5; 2.7–500), “broad-spectrum antibiotic use within the 3 months previous to admission” (16.4; 1.63–166.7), “COPD exacerbation when Aspergillus was isolated” (56.2; 2.7–1,000), and “antifungal therapy with a drug other than voriconazole” (46.5; 3.2–668.1). A total of 27 (47.3%) patients received voriconazole (alone [n = 13] or in combination [n = 14]), and 30 (52.7%) patients received other antifungals. Both groups were comparable, but survival was higher in patients treated with voriconazole (n = 13) than in patients receiving other antifungals (n = 3) (48% vs. 10%; P = 0.002). There were no differences between the number of days of therapy from the isolation of Aspergillus to initiation of antifungal treatment in patients with poor and good outcome (4.06 vs. 3.56; P = 0.634).

**Conclusions:** Patients with COPD and IPA had a high mortality rate, above 70%, particularly in patients with a worse clinical condition. Although our series included a limited number of cases, antifungal therapy with voriconazole in patients with COPD and IPA had a better outcome. J. Guinea is contracted by FIS CM05/00171; M. Torres-Narbona is contracted by FIS CM08/00277.

**P1748** Voriconazole-impregnated beads in the treatment of candidal prosthetic joint infection

D. Harvey, J. Tomlinson, A. Cooper, S. Buckley, R. Townsend, R. Kerry, D. Oliver (Sheffield, Bristol, UK)

**Objectives:** Below we report on the use of voriconazole-loaded bone cement and its elution data in salvage therapy in 2 cases of candidal prosthetic hip infection.

**Methods:** Candida tropicalis and C. albicans respectively were cultured after aseptic aspiration under radiological guidance of two patients with infected hip prostheses. As part of a 2-stage revision procedure, voriconazole beads were inserted at the time of the first stage. 1 gram of voriconazole powder was mixed with the bone cement to make the beads. Deep drains were left in situ for 48 hours. The drain volume and voriconazole concentration as well as serum voriconazole concentration were recorded at intervals for 48 hrs. The use of prolonged intravenous antifungal therapy was avoided.

**Results:** The table shows high local voriconazole concentrations in the drain and thus by inference at the operative site, which reduce over 48hrs but still remain relatively high. Furthermore there was no detectable serum voriconazole in the first patient and minimal levels in the second patient.

**Conclusions:** Fungal prosthetic joint infections, whilst rare, provide a difficult treatment challenge. Excision arthroplasty and prolonged intravenous antifungal agents appear to be the mainstay of therapy. Previously our unit has reported the successful use of fluconazole impregnated cement beads to eradicate C. albicans and C. parapsilosis joint infection. Fluconazole is no longer available in powdered form thus this option is not available. Voriconazole is available in powdered form and is thermostable which makes it suitable for incorporation in polymethylmethacrylate beads. Using this method the data presented show very high local concentrations of voriconazole are achieved which would be expected to sterilise the operative site, whilst avoiding any potential systemic side effects of voriconazole.

Long-term follow-up data is awaited. In conclusion we present a potential therapeutic option for the treatment of candidal prosthetic joint infections.

<table>
<thead>
<tr>
<th>Location</th>
<th>Voriconazole concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time since operation (hrs)</td>
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</tr>
<tr>
<td>Patient 1</td>
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<tr>
<td>Serum</td>
<td>0</td>
</tr>
<tr>
<td>Drain</td>
<td>306</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>0.6</td>
</tr>
<tr>
<td>Drain</td>
<td>n/a</td>
</tr>
</tbody>
</table>

**P1749** Voriconazole plasma levels monitoring in haematologically oncological patients

M. Tóskocárová*, J. Wintourcová, Z. Rácl, L. Malášková, I. Kocmanová, M. Lengerová, B. Weinbergerová, J. Mayer, K. Hrnčirotová (Brno, CZ)

**Objectives:** Voriconazole plasma levels measurement enables to optimise dosing and improves the efficacy of antifungal treatment.

**Methods:** Retrospective analysis of documentation and laboratory results of patients treated with voriconazole from August 2005 to November 2008 was performed. Steady-state plasma voriconazole levels were obtained using a high-performance liquid chromatography assay.

**Results:** 618 plasma samples from 112 patients were analyzed; 1–29 samples per patient. 45.5% (n = 51) patients were after allogeneic haematopoietic stem cell transplantation. Voriconazole was administered in 43% as prophylaxis, in 11% as an empirical antifungal treatment and in 46% as a preemptive treatment of invasive fungal infection. In 105 patients (93.7%) voriconazole was administrated orally, only in 7 cases
Therapeutic drug monitoring of posaconazole in 54 adult lung transplant patients


Aspergillus is a major complication in lung transplantation (LTx) particularly in case of cystic fibrosis (CF). Posaconazole (PSZ) is indicated for the curative and prophylactic treatment of invasive fungal infections using respectively 400 mg×2/day and 200 mg×3/day doses. PSZ absorption is saturable, therefore dose increase often needed in CF patients is limited. PSZ half life is long (35 h), resulting in long time to steady state (SS).

Objective: To show that PSZ therapeutic drug monitoring (TDM) in CFLTx allows 1. the achievement of trough concentrations (C0) levels consistent with efficiency (>0.5 mg/L) or at least detectable (>0.2 mg/L); 2. the management of PSZ metabolic drug interactions with immunosuppressants.

Methods: Retrospective and prospective cohort of CFLTx under PSZ between 2006 and 2008 in our centre. Longitudinal collection of both doses and C0 (at SS) data for PSZ and immunosuppressants. TDM by determination of plasma PSZ by LC assay with fluorimetric detection.

Results: 17 CFLTx, aged 26±8 years, received curative (n=2), preemptive (n=14) or prophylactic (n=1) PSZ treatment. Caspofungin was combined to PSZ in 8 patients. The mean treatment duration was 228±197 days, [14–621]. PSZ introduction corresponded to immediate (n=4), first year (n=5) or beyond (n=8) postTx. 220 PSZ C0 were analyzed. No post Tx time effect on PSZ exposure was observed. The mean PSZ C0 was 0.7±0.5 mg/L, [0.2;1.6] using an average PSZ dose of 108±310 mg/day, [73;1889], resulting in more than +35% of the recommended dose (p<0.001). In 65% of patients, a dose adjustment was required on day 11 as an average. Such adjustment has been successful in 86% of cases. PSZ was withdrawn (n=8) because of 7 negative cultures and 1 intravenous route (posaconazole + caspofungin). No particular adverse event (gastrointestinal disorders, headache) has been recorded during PSZ courses. The immunosuppressant tacrolimus dose was tapered by a factor 3 during the co-prescription with PSZ.

Conclusion: PSZ TDM was useful to achieve PSZ therapeutic C0 in CFLTx. Despite increase and/or split dose, no C0 >3 mg/L has been observed. PSZ safety profile was good. Indeed, PSZ acted as a moderate CYP3A4 metabolic inhibitor, justifying a joint TDM of both PSZ and immunosuppressants to manage the immunosuppressants dose adjustment at the introduction or discontinuation of PSZ.

P1752 Salvage therapy with posaconazole and topical amphotericin B: an unusual case of Aspergillus fumigatus empyema and bronchopleural fistulae after extrapleural pneumonectomy and chemo-radiotherapy

A. Galeri, M. Purshott, J. Zacharias, J. Paterson* (Blackpool, UK)

Background: Malignant mesothelioma, an aggressive malignancy, has a median survival of 14-months after onset of symptoms. A combination of chemo-radiotherapy and extrapleural pneumonectomy [CREPP], recommended treatment, is still associated with 4−15% mortality and a 62% complication rate.

Having found no similar report in literature, we believe this is first case of aspergillus fumigatus [AF] empyema and bronchopleural fistulae after CREPP, and treated successfully with systemic and topical antifungal therapy and went on to have his definitive surgical procedure.

Case study: A 63-yr-male was hospitalised with chest symptoms 2-weeks post chemoradiotherapy following right extra-pleural pneumonectomy for malignant epithloid mesothelioma in November 2006. CT scan and bronchoscopy revealed bronchopleural fistula in rt pleural cavity. He underwent rib-resection to permit drainage of empyema. He was treated with multiple broad spectrum antibiotics matching positive bacterial cultures. Multiple cultures of AF and a visible growth of gray-cottony growth lining mesh [dome diaphragm]; prothetic patch/pericardium and chest cavity. Voriconazole followed by caspofungin failed to clear fungus [figure 1]. His further surgical management was held up in presence of fungus. A salvage therapy, after exhausting all options and with patient consent – a paste-mixture of Spongostan [a topical haemostat in surgery] and amphotericin B applied to the whole of right pleural cavity;prothetic sheaths replacing the pericardium and diaphragm, under general anaesthesia and rib resection was fashioned. Oral posaconazole was given for 6-weeks. Following multiple negative cultures, the patient went on to have planned surgery and closure of his chest defect.

**P1750** Therapeutic drug monitoring of posaconazole in 54 adult patients


Objective: To assess the prevalence of low posaconazole (PSZ) plasma levels (PPL) in case of prophylactic or curative treatment, and host factors associated with low PPL.

Methods: We retrospectively reviewed all adult patients who underwent measurement of PPL after at least 5 days of treatment between 2006 and 2008 at Necker-Enfants malades university hospital. Therapeutic drug monitoring (TDM) was performed by high-performance chromatography and ultra-violet detection. Clinical and biological data were obtained at the initiation of PSZ. Low PPL was defined as a concentration lower than 500 ng/ml (Andes et al., AAC January 2009).

Results: 54 patients were included: 36 receiving prophylactic (200 mg t.i.d.) [allogenic bone marrow transplantation (75%), haematological malignancy with prolonged neutropenia (19%) or constitutive immunodeficiency (6%) and 18 curative posaconazole therapy (400 mg b.i.d.). Prevalence of low PPL was 16/36 (44%) in the prophylactic group and 22/4/16) in the curative treatment group.

In the prophylactic group, low PPL tended to be more frequent in case of digestive disease (62% versus 30%, p = 0.051) and was significantly more frequent in patients with diarrhoea (71% versus 24%, p = 0.009) or mucositis (100% versus 33%, p = 0.004).

In the prophylactic group, only 2 patients experienced IFI and both exhibited a low PPL. The only adverse event was hepatotoxicity in 2/54 patients (3.7%).

Conclusions: Low PPL is common, significantly more frequent in case of diarrhoea or mucositis and potentially associated with the subsequent occurrence of IFI. PSZ TDM is therefore mandatory in immunosuppressed adults.

**P1751** Posaconazole therapeutic drug monitoring in cystic fibrosis lung transplant patients


Aspergillus is the major complication in lung transplantation (LTx) in case of cystic fibrosis (CF). Posaconazole (PSZ) is indicated for the curative and prophylactic treatment of invasive fungal infections using respectively 400 mg×2/day and 200 mg×3/day doses. PSZ absorption is saturable, therefore dose increase often needed in CF patients is limited. PSZ half life is long (35 h), resulting in long time to steady state (SS).

Objective: To show that PSZ therapeutic drug monitoring (TDM) in CFLTx allows 1. the achievement of trough concentrations (C0) levels consistent with efficiency (>0.5 mg/L) or at least detectable (>0.2 mg/L); 2. the management of PSZ metabolic drug interactions with immunosuppressants.

Methods: Retrospective and prospective cohort of CFLTx under PSZ between 2006 and 2008 in our centre. Longitudinal collection of both doses and C0 (at SS) data for PSZ and immunosuppressants. TDM by determination of plasma PSZ by LC assay with fluorimetric detection.

Results: 17 CFLTx, aged 26±8 years, received curative (n=2), preemptive (n=14) or prophylactic (n=1) PSZ treatment. Caspofungin was combined to PSZ in 8 patients. The mean treatment duration was 228±197 days, [14–621]. PSZ introduction corresponded to immediate (n=4), first year (n=5) or beyond (n=8) postTx. 220 PSZ C0 were analyzed. No post Tx time effect on PSZ exposure was observed. The mean PSZ C0 was 0.7±0.5 mg/L, [0.2;1.6] using an average PSZ dose of 108±310 mg/day, [73;1889], resulting in more than +35% of the recommended dose (p<0.001). In 65% of patients, a dose adjustment was required on day 11 as an average. Such adjustment has been successful in 86% of cases. PSZ was withdrawn (n=8) because of 7 negative cultures and 1 intravenous route (posaconazole + caspofungin). No particular adverse event (gastrointestinal disorders, headache) has been recorded during PSZ courses. The immunosuppressant tacrolimus dose was tapered by a factor 3 during the co-prescription with PSZ.

Conclusion: PSZ TDM was useful to achieve PSZ therapeutic C0 in CFLTx. Despite increase and/or split dose, no C0 >3 mg/L has been observed. PSZ safety profile was good. Indeed, PSZ acted as a moderate CYP3A4 metabolic inhibitor, justifying a joint TDM of both PSZ and immunosuppressants to manage the immunosuppressants dose adjustment at the introduction or discontinuation of PSZ.
He made good recovery from this complication but subsequently succumbed to mesothelioma after 8 months.

**Discussion:** This case of AF empyema and bronchopleural fistulae following CREPP was complicated by local fungal colonisation/infection. This fungal presence held up his further surgical management. Systemic antifungals failed to clear the relatively avascular fibrosed chest cavity with extensive prosthetic material. Spongostan mixed with amphotericin B to allow sustained exposure at this complex anatomic site and posaconazole as systemic therapy. Intra-pleural irrigation with amphotericin B in Aspergillus Emypema is reported but would have not provided sustained local exposure in this unusual case. Video/picture to be presented.

**Conclusions:**

Febrile neutropenia is the major complication in neutropenic patients with haematological malignancies. Preliminary report of a Spanish multicentre study

**Background:**
The aim of this study was to assess the principal “microbiological” features and clinical presentation of the patients with a haematological malignancy (HMP) admitted in an ICU due to a severe infection and specially to analyse the main aetiologies of these infections in order to avoid an excessive rate of inadequate empirical antibiotic treatment in these patients.

**Methods:**
This prospective observational multicentre study was conducted at 30 sites in Spain from June 2006 through October 2008. Any patient with haematological admitted in ICU was potentially eligible. Clinical and microbiological features were recorded. A preliminary descriptive analysis was performed.

**Results:**
Among 237 HMP admitted in an ICU, 154 of them (64.9%) presented a severe infection as a cause of admission. Relation men:women was 1.2. The age of patients was 54.17±16.65 years. APACHE II score was 23.8±8.3. Septic shock was the most frequent clinical presentation (58.5%). The principal origin of infections were: respiratory (59.7%), abdominal (14%) and unknown (16.6%).

The majority of episodes were nosocomial acquired (63%). The most frequent severe infections involved were: Nosocomial pneumonia (28.6%), community acquired pneumonia (26.4%) primary bacteraemia (10%) and peritonitis (5.7%).

Associated bacteraemia was present in 49.3% of infected HMP. The most frequently microorganism isolated were: Escherichia coli (17.9%), Streptococcus pneumoniae (10.3%), Acinetobacter baumannii (6.6%), Klebsiella pneumoniae (6.6%) and Staphylococcus aureus (5.6%).

The incidence of fungal infections was also high; Candida spp. was isolated in 2.8% of the infected HMP and proven or probable invasive Aspergillosis was considered in 5.1% of infected HMP. Pseudomonas aeruginosa (13.2%) and Acinetobacter baumannii (6.6%) were the most frequent microorganisms isolated in respiratory samples.

**Conclusions:**
Severe infection is the main cause of admission in ICU of HMP. The most common clinical presentation still is septic shock secondary to pulmonary foci. Nowadays potential multidrug resistant microorganism such as Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae and Staphylococcus aureus as well as invasive fungal infections should be keep in mind in order to avoid inadequate empirical antibiotic therapy in HMP admitted in ICU due to a severe infection.

**Infections in the immunocompromised host**

**P1753** Febrile neutropenia in patients with haematological malignancies


**Aim:** The aim of this study was to evaluate the incidence of infection in neutropenic patients with haematological malignancies in a prospective study in 7 haematological centres.

**Patients/methods:** We enrolled neutropenic patients (neutrophil count, ANC <500/mm³) due to chemotherapy. We recorded: fever >38°C, disease, chemotherapy, duration of neutropenia, growth factor (G-CSF), chemoprophylaxis, antibiotics, conditions of hospitalisation, Central Venus Catheter (CVC), isolated bacteria and resistance to antibiotics.

**Results:** We studied 892 cases of neutropenia in 581 patients. In 305 cases there was a CVC. G-CSF was administered in 725 cases. Chemoprophylaxis was administered in 510 cases. The median duration of neutropenia was 14 days. In 672 cases, one or more fever waves have been observed. 51 patients died due to the infection (8.8%). The existence of CVC was significant for the incidence of fever (85% vs 70% p <0.01) while the administration of G-CSF was not (74% vs 79%, p=ns). The nadir of the ANC was statistical significant (84.4% for ANC <100/mm³ vs 53.5% for ANC >100/mm³, p <0.01). Bacteria were isolated in 559 of the febrile cases (62%). The ratio of Gram(+) to Gram(−) bacteraemia to Gram(−) was 10 to 6. 30.6% of Enterococci were resistant to glycopeptides. The percentage of resistance of *Pseudomonas* sp. in carbapenems was also high (50%). Resistance of Enterobacteriaceae to carbapenems is alarmingly increasing (8–12%).

**Conclusions:**
Febrile neutropenia is the major complication in neutropenic patients with haematological malignancies. Our data showed a statistical significant difference for patients with CVC and with ANC <100/mm³. There was no statistical significant difference for patients receiving G-CSF, chemoprophylaxis and there was no correlation to the duration of neutropenia and to HEPA filter rooms. The mortality due to infection was low.

**P1754** Updated epidemiology of severe infections as a cause of admission in an intensive care unit in patients with haematological malignancies. Preliminary report of a Spanish multicentre study


**Background:**
Infections in the immunocompromised host

**Methods:**
This prospective observational multicentre study was conducted at 30 sites in Spain from June 2006 through October 2008. Any patient with haematological admitted in ICU was potentially eligible. Clinical and microbiological features were recorded. A preliminary descriptive analysis was performed.

**Results:**
Among 237 HMP admitted in an ICU, 154 of them (64.9%) presented a severe infection as a cause of admission. Relation men:women was 1.2. The age of patients was 54.17±16.65 years. APACHE II score was 23.8±8.3. Septic shock was the most frequent clinical presentation (58.5%). The principal origin of infections were: respiratory (59.7%), abdominal (14%) and unknown (16.6%). The majority of episodes were nosocomial acquired (63%). The most frequent severe infections involved were: Nosocomial pneumonia (28.6%), community acquired pneumonia (26.4%) primary bacteraemia (10%) and peritonitis (5.7%). Associated bacteraemia was present in 49.3% of infected HMP. The most frequently microorganism isolated were: *Escherichia coli* (17.9%), *Streptococcus pneumoniae* (10.3%), *Acinetobacter baumannii* (6.6%), *Klebsiella pneumoniae* (6.6%) and *Staphylococcus aureus* (5.6%). The incidence of fungal infections was also high; *Candida* spp. was isolated in 2.8% of the infected HMP and proven or probable invasive Aspergillosis was considered in 5.1% of infected HMP. *Pseudomonas aeruginosa* (13.2%) and *Acinetobacter baumannii* (6.6%) were the most frequent microorganisms isolated in respiratory samples.

**Conclusions:**
Severe infection is the main cause of admission in ICU of HMP. The most common clinical presentation still is septic shock secondary to pulmonary foci. Nowadays potential multidrug resistant microorganism such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Staphylococcus aureus* as well as invasive fungal infections should be keep in mind in order to avoid inadequate empirical antibiotic therapy in HMP admitted in ICU due to a severe infection.

**P1755** Differences in development of catheter-related infections in critically ill patients

**E. Chiono*, E. Dalla, Z. Chiolou, A. Georgoulis, P. Giannakakos, E. Skouteli (Athens, GR)

**Objectives:**
To study and compare some characteristics of infections associated with central venous catheters in patients hospitalised in the Intensive Care Unit (ICU) and Oncology Units (OUs) of our hospital.

**Methods:**
During one year period (2008) we studied 118 patients from a seven bed ICU (60 patients) and two oncology units (58 patients) for catheter related infections. The catheter tips were cultured using the BacT-Alert automated system for 7 days.

**Results:**
We cultured 150 central iv catheters, used for parenteral nutrition and drug administration. The preferred vein in ICU and OUs...
Sepsis in the elderly: different?

J. Nikhilesh*, S. Ratan (Indore, IN)

Objective: To analyze outcomes in patients with sepsis in geriatric vis-à-vis non geriatric cohorts admitted in critical care unit (CCU) and the epidemiological profile for organisms leading to same

Methods: Patients presenting with sepsis were enrolled and divided into a control group (<65 years) and test group (>65 years). Data collection was done with a focus on demographics, SOFA scores, requirement of mechanical ventilation (MV), requirement of renal replacement therapy (RRT), co-morbidities (Diabetes mellitus [DM], Hypertension [HTN], coronary artery disease [CAD]) and length of stay in CCU (LOS). A set of paired blood cultures were collected at baseline from each patient. Discharges from CCU/death were considered as end points.

Results: Sixty five patients (n=65, M: F-44:21, Age-42.5 ± 8.6 years) in control group and seventy four patients in test group (n=74, M:F = 52:22, Age 72.7±6.3 years) were enrolled (Jan 2007–Dec 2008).

<table>
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<th>Variable</th>
<th>Control</th>
<th>Test</th>
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<td>DM</td>
<td>32.3% (n=21)</td>
<td>44.6% (n=33)</td>
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<td>HTN</td>
<td>38.5% (n=25)</td>
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<td>Culture positive</td>
<td>40% (52/130)</td>
<td>44.6% (66/148)</td>
</tr>
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</table>

The organisms grown in cultures were MSSA (n=10), MRSA (n=14), Klebsiella [ESBL] (n=19), E. coli [ESBL] (n=3) and Acinetobacter (n=6) and MRSA and E. coli [ESBL] (n=12), Klebsiella [ESBL] (n=26), E. coli (n=9), Pseudomonas (n=7) and Acinetobacter (n=6) respectively for test group. Logistic regression revealed MV in control group and MV, RRT in test group were independent predictors of mortality.

Conclusion: Prevalence of ESBLs and Pseudomonas is more in geriatric groups vis-à-vis non-geriatric cohorts and requirement of MV and RRT in geriatric groups confers an additional risk of mortality. However, owing to small sized samples it would be difficult to interpret the above findings on a generalised basis and we would need bigger samples and more epidemiological studies to confirm the same.

Infections in the immunocompromised host

P1756 The production of ESBLs does not affect the early and late clinical outcome of E. coli or K. pneumoniae bacteremia in febrile neutropenia


Objectives: Gram-negative bacilli (GNB) such as E. coli and Klebsiella species are main pathogen in febrile neutropenia even if the proportion of Gram-positive cocci is increasing. GNB producing extended-spectrum β-lactamases (ESBLs) are an emerging problems in nosocomial infection. Nevertheless, information about risk factors and clinical outcomes of ESBLs-producing GNB bacteremia is limited in febrile neutropenia.

Methods: We retrospectively reviewed medical records and analyzed patients' characteristics, risk factors and outcomes of ESBLs-producing E. coli and K. pneumoniae bacteremia compared with non-ESBLs-producing strains in febrile neutropenia at the Catholic HSCT centre from Jan 2005 to Dec 2006.

Results: In a total of 101 isolates of E. coli or K. pneumoniae bacteremia in febrile neutropenia, ESBLs and non-ESBLs were 25 and 76, respectively. Production of ESBLs were more common in K. pneumoniae (10/14) than E. coli (15/87) (P<0.001). Age, sex, underlying disease, and type of co-morbidity of two groups were not different. Mean hospital stay before bacteremia were longer in ESBLs group (27±14 vs 15±5 days, P<0.001). Number and total duration of admission during prior 12 months did not differ in two groups, but care in intensive care unit (ICU) was more frequent in ESBLs group (20% vs 3%, P=0.004). There was no difference in exposure to antibiotics and total duration of antibiotics administration during prior 90 days between the two groups. Duration of febrile neutropenia was longer in ESBLs group (5±5 vs 2±2 days, P<0.001). Intravenous antifungal therapy and granulocyte transfusion were applied more frequently in ESBLs group (76% vs 47%, P=0.027, 28% vs 8%, P=0.013, respectively). But there was no difference in SAPS-II score, total duration of IV antibiotics, length of hospitalisation, and mortality rate at 7-, 30-day, and last visit between the two groups.

Conclusion: Prior care in ICU and duration of hospital stay were risk factors for acquisition of ESBLs-producing GNB. Despite ESBLs-producing GNB bacteremia was associated with longer duration of febrile neutropenia and so more frequent use of IV antifungals, clinical outcomes showed no difference between ESBLs and non-ESBLs groups. Decision of empirical antibiotics in febrile neutropenia should be made carefully under consideration of presence of ICU care, recent hospital stay, and prolonged febrile neutropenia.

P1757 Taxonomic structure of anaerobic bacteria in cancer patients in a Russian cancer research centre

I. Shinklova*, N. Dmitrievsca (Moscow, RU)

Objective: To determine the frequency of isolation and spectrum of anaerobic bacteria in cancer patients within the 6-year period (2003–2008).

Methods: Anaerobic bacteria isolated from various clinical specimens were analysed. They were obtained from surgical wounds after orthopaedic operations, pleuropulmonary, abdominal, biliary tract and genitourinary infections and bacteraemia. Pus, body fluids, discharges from drain pipes, bile and the pieces of tumour tissues were the most common clinical material used. Clinical samples were transported within 2h of collection and special anaerobic transport systems were not used. Cultivation methods included enrichment media and GasPak jar. Isolates were identified by the BBL Crystal anaerobes identification system, API System rapid ID 32 A, and routine methods.

Results: The total number of positive cultures including both aerobes and anaerobes was 1303 out of 2384 (54.6%). Anaerobes were detected in 186 samples, in 139 (74.7%) of them grew both aerobic and anaerobic bacteria, and in 47 (25.3%) cultures only anaerobes were found. A monocultures or mix of anaerobes, without facultative and aerobic
bacteria, were isolated from aspirates from surgical wounds (38%), abscesses (22%), blood (21%) and body fluids (19%). A total of 259 strains of anaerobic bacteria were isolated: Lactobacillus – 59(22.8%), Gram(+) cocci – 39(15.1%), Gram(–) rods – 39(15.1%), Veillonella – 27(10.4%), Propionibacterium acnes – 27(10.4%), Actinomyces – 16(10.0%), Clostridium – 25(9.7%), other anaerobes – 17(6.5%). The majority of anaerobic bacteria were recovery from patients with stomach cancer followed by pancreas cancer, liver and biliary tract cancer and also bone cancer. Lactobacillus spp. are usually isolated from the pieces of neoplastic tissues of oral cavity and oesophagus, collected at the time of surgery, and very often from bile. Although Lactobacillus spp., Propionibacterium acnes and Veillonella spp. are considered to be of low virulence, they may cause a serious infections in immunocompromised patients with malignancies.

Conclusion: Taxonomic structure of anaerobic bacteria in cancer patients have specific differences. Anaerobic infections arise from the endogenous flora, provided that appropriate host and environmental factors are present. One of the underlying conditions to anaerobes proliferation is malignancies, often associated with surgery, chemotherapy and radiation therapy, when the host immune response is compromised.

### P1750 Infections caused by Gram-positive anaerobic cocci in cancer patients

I. Shilnikova*, N. Dmitrieva (Moscow, RU)

**Objective:** Gram-positive anaerobic cocci (GRAP) originally classified in the genus Peptostreptococcus are clinically significant organisms often isolated from mixed infections. The aim of present study was to determine the frequency of GRAP in cancer patients, their isolation sites and the susceptibility to antibiotics in N.N.Blokhin Cancer Research Center of Russia.

**Methods:** Data on GRAP isolated from various clinical specimens of cancer patients were analysed. Aspirates from surgical wounds, pus of abscesses, body fluids and the pieces of tumour tissues were transported within 2h of collection without using special anaerobic transport systems and cultivated in Schaedler's agar and Thioglycolate broth enriched with hemin and menadione. The cultures were incubated anaerobically using the GasPak jars (Oxoid) at 37°C for 48–96h and identified biochemically by using a combination of conventional tests and the commercial kits Rapid ID 32A (BioMerieux) and Crystal Anaerobe ID (Becton Dickinson).

**Results:** In total, 39 strains of GRAP were isolated from 34 patients: Finegoldia magna – 17 (44%), Peptostreptococcus asaccharolyticus – 10 (26%), Peptostreptococcus micros – 8 (20%) and Peptostreptococcus anaerobius – 4 (10%). Sources of their isolation were head and neck region (5 species), lungs (6), intra-abdominal area (6), women's reproductive system (7), soft tissue (9), bone and joint (prosthetic infection, 6). All isolates showed high susceptibility to penicillins, metronidazole, chloramphenicol and doxycycline. Over 50% F magna strains were resistant to clindamycin, cefotaxime and ceftriaxone; 30–40% – to fluoroquinolones, including moxifloxacin, and 60–70% – to azithromycin and erythromycin. In P. anaerobius strains only resistance to fluoroquinolones (33%) was found. P. asaccharolyticus strains demonstrated intermediate resistance to azithromycin (22%) and erythromycin (56%) and resistance to ciprofloxacin (44%), but all strains were susceptible to levofloxacin and moxifloxacin. In contrast to F magna, P. anaerobius and P. asaccharolyticus were susceptible to clindamycin and cefalosporins.

**Conclusion:** Gram-positive anaerobic cocci are frequently isolated from a wide range of body sites and associated with serious infections in cancer patients.

### P1760 Infectious complications in cancer patients treated with dasatinib (BMS-354825)

S. Ahmed, G. Rodriguez, S. Ejaz, F. Al Akhrass, A. Safdar * (Houston, US)

**Background:** Tyrosine kinase inhibitors (TKI) interrupt T-cell receptor mediated T-cell proliferation, activation and selective inhibition of memory CTL responses without affecting primary T or B cell responses. Few cases of cytomegalovirus (CMV), varicella-zoster virus (VZV) and Parvovirus B19 infections have been reported during dasatinib therapy.

**Objective:** To determine the frequency of GRAP in cancer patients, their isolation sites and the susceptibility to antibiotics in N.N.Blokhin Cancer Research Center of Russia.

**Methods:** Aspirates from surgical wounds, pus of abscesses, body fluids and the pieces of tumour tissues were transported within 2h of collection without using special anaerobic transport systems and cultivated in Schaedler's agar and Thioglycolate broth enriched with hemin and menadione. The cultures were incubated anaerobically using the GasPak jars (Oxoid) at 37°C for 48–96h and identified biochemically by using a combination of conventional tests and the commercial kits Rapid ID 32A (BioMerieux) and Crystal Anaerobe ID (Becton Dickinson).

**Results:** In total, 39 strains of GRAP were isolated from 34 patients: Finegoldia magna – 17 (44%), Peptostreptococcus asaccharolyticus – 10 (26%), Peptostreptococcus micros – 8 (20%) and Peptostreptococcus anaerobius – 4 (10%). Sources of their isolation were head and neck region (5 species), lungs (6), intra-abdominal area (6), women's reproductive system (7), soft tissue (9), bone and joint (prosthetic infection, 6). All isolates showed high susceptibility to penicillins, metronidazole, chloramphenicol and doxycycline. Over 50% F magna strains were resistant to clindamycin, cefotaxime and ceftriaxone; 30–40% – to fluoroquinolones, including moxifloxacin, and 60–70% – to azithromycin and erythromycin. In P. anaerobius strains only resistance to fluoroquinolones (33%) was found. P. asaccharolyticus strains demonstrated intermediate resistance to azithromycin (22%) and erythromycin (56%) and resistance to ciprofloxacin (44%), but all strains were susceptible to levofloxacin and moxifloxacin. In contrast to F magna, P. anaerobius and P. asaccharolyticus were susceptible to clindamycin and cefalosporins.

**Conclusion:** Gram-positive anaerobic cocci are frequently isolated from a wide range of body sites and associated with serious infections in cancer patients.

**Methods:** This retrospective analysis of infections in 57 patients during dasatinib therapy alone or in combination with other antineoplastic regimens during May 2006 through December 2007 was performed after obtaining IRB approval. The values are presented as median ± s.d. Categorical data was analyzed using Chi-square.

**Results:** Forty-two episodes of infection were identified in 28 patients (49%) including 19 episodes (42%) during neutropenia; please refer to the table below. Duration of chemotherapy was 135±191 days in patients who developed infection vs. 228±347 days in who no infection occurred. There were no significant differences in age, race or prior haematopoietic stem cell transplantation in patients who developed infection vs. patients with no infection (n = 29; 51%). In patients who developed infections vs. who did not, had more co-morbidities including diabetes (32% vs. 14%; P = 0.04); had Ph+ acute lymphocytic leukaemia (ALL; 39% vs. 10%; P = 0.01); had received high-dose steroids (50% vs. 21%; P = 0.02), and frequently received dasatinib with another antineoplastic agent(s) (54% vs. 28%; P = 0.04). Dasatinib was discontinued due to adverse events in 24% of patients with infection vs. 16% with no infection. Overall mortality was higher in patients with infection (61%) compared with 24% in patients with no infection (P = 0.08). In only one patient infection was considered as the cause of death.

**Conclusions:** Infections were common in patients receiving dasatinib therapy and significantly more frequently seen in patients with ALL being treated with steroids and multiple antineoplastic agents.

| Table. Type of infection episodes in 26 patients during dasatinib therapy |
|--------------------------|------------------|
| Infection episodes        | n (%)            |
| Total infection episodes  | 42 (100)         |
| Clinical documented infections | 23 (55)        |
| Clinical pneumonia (bacterial) | 11 (26)       |
| Possible fungal pneumonia | 4 (10)           |
| Soft tissue infection*     | 3 (7)            |
| Gastroenteritis/mucositis** | 3 (7)           |
| Febrile neutropenia (cause not identified) | 1 (2)        |
| Urinary tract infection   | 1 (2)            |
| Microbiological documented infections | 19 (45)   |
| Gram-positive bacterial infections | 12 (29)  |
| Clostridium difficile colitis | 14 (30)       |
| CoNS CrBSI                | 1 (2)            |
| Enterococcus CrBSI        | 2 (5)            |
| MRSA CrBSI                | 2 (5)            |
| MRSA otitis externa       | 1 (2)            |
| Gram-negative bacterial infections | 4 (10) |
| Klebsiella spp. neutropenic enterocolitis | 2 (5) |
| Pseudomonas spp. CrBSI    | 1 (2)            |
| Pseudomonas spp.-Klebsiella spp. urinary tract infection | 1 (2) |
| Viral infections          |                  |
| RSV pneumonia             | 1 (2)            |
| HSV oral labial           | 1 (2)            |
| Fungal infection          |                  |
| Candida krausei CrBSI     | 1 (2)            |

*2 cellulites/1 perirectal abscess; **1 neutropenic enterocolitis.
CrBSI: catherer-related bloodstream infection; CoNS: coagulase-negative staphylococci; MRSA: methicillin resistant Staphylococcus aureus.
Infections in the immunocompromised host

**P1761** Biomarkers of infection and septic shock in neutropenic patients

R. Jedd†, R. Ben Amor, L. Aissoua, L. Thabet, K. Kacem, W. Bouteraëa, H. Ben Abid, Z. Bel Hajjali, B. Meddeb (Tunis, TN)

Procalcitonin and C-reactive protein are the most markers of inflammation used in the diagnosis of infection. We aimed at evaluating the diagnostic and prognostic value for infection of semi-quantitative PCT (>0.5, >2 and >10ng/ml), CRP (N < 6mg/L), serum bicarbonate (N: 24–26mmol/L), serum lactate (N < 2.2mmol/L) and phosphataemia (N:0.8–1.4mmol/L).

50 neutropenic febrile episodes were noted among 27 patients with acute leukaemia. All patients presented neutropenia (ANC < 0.5 × 10^9/L) lasting for more than 7 days. They were on oral anti-bacterial (polymyxin B, gentamicin) and anti-fungal (fungizone) prophylaxis. The first neutropenic febrile episode was treated with Piperacillin-Tazobactam and Polymyxin B IV, if the patient remain febrile at 48 hour from the start of this first line the fungizone iv is added. Impenem is introduced in case of non response and finally glycopeptides are introduced according to the IDSA criteria [1]. Severe sepsis and septic shock are defined according to the criteria of the consensus conference of the ACCP/SCCM [2].

Consecutive sample for PCT (semi-quantitative test, BRAHAMS), CRP, Phosphataemia, Serum lactate, and serum bicarbonate were measured at each neutropenic febrile episode. Seven episodes (14%) were clinically documented: pneumonia (4), neutropenic enterocolitis (1), mucositis (1) and skin infection (1). Microbiologically documented infection (30%) were due to 9 Gram− and 6 Gram+. Klebsiella (7), Acinetobacter (1), E. coli (1), Staphylococcus (6). Fever of unknown origin accounted for the remaining 28 febrile episodes.

6 episodes (12%) were complicated with septic shock, with infection related mortality of 18.5% (5/27 patients). PCT > 0.5ng/ml was noted in 18 (36%) febrile episodes, whereas high level (>10ng/ml) are noted in 11 episodes. Median level of each biomarker was: CRP 88.8mg/L (range, 2.2–183mg/L), Serum lactate 2.3mmol/L (range, 0.8–3.3mmol/L), Serum bicarbonate 24.3mmol/L (range, 9.4–32.1mmol/L), and phosphataemia 1.08mmol/L (range, 9.4–32.1mmol/L).

PCT > 0.5 ng/ml (p = 0.004; OR = 6.6) and CRP > 100 ng/ml (p = 0.008; OR = 6.1) were correlated with microbiologically and/or clinically documented infection. CRP > 100ng/mL correlates with Gram− infection (p = 0.045). PCT > 10ng/ml (p = 0.017; OR = 10.5) and serum lactate >3mmol (p = 0.04; OR = 16) are associated with occurrence of septic shock.

Several level of PCT correlated with infection and severity and is useful to monitor in neutropenic setting.

**Reference(s)**


**P1762** Can multiplex PCR (SeptiFast) detect DNAemia before occurrence of sepsis in neutropenic patients?


**Objectives**: Invasive infections are the leading cause of morbidity and mortality in patients treated for haematological malignancies. Blood cultures are often negative in neutropenic patients because of low-burden of organisms, previous antibiotic therapy or non-infectious reason of fever. More rapid, accurate and sensitive diagnostic tools are needed. We assessed the multiplex real-time PCR SeptiFast (Roche Diagnostics) to detect microbial DNA in neutropenic patients without fever.

**Methods**: We prospectively included all patients aged ≥18 years hospitalised between 01/2007 and 12/2008 in the bone marrow transplant unit at our institution for an autologous or allogeneic haematopoetic stem cells transplantation or induction/consolidation chemotherapy. 1.5 ml of EDTA-blood was routinely collected for SeptiFast at admission and thereafter 3×/week (Mo, We, Fr) until discharge or recovery from neutropenia (>0.5 × 10^9/L neutrophils over >3 days). In addition, blood was collected for SeptiFast simultaneously with blood cultures at new appearance or persistence of fever despite antibiotic treatment of >72h.

**Results**: During the study period, 875 SeptiFast tests were performed in 82 patients (median age, 47 y; range 18–80 y; 54% males). Overall, 102 episodes of neutropenia occurred in 82 patients with a median duration 14 d (± 1428 neutropenic days). The haematological diseases included acute myeloc leukaemia (44%), acute lymphatic leukaemia (16%), non-Hodkin lymphoma (8%), chronic myeloid leukaemia (7%), myelodysplastic syndrome (6%) and other (19%). SeptiFast was positive in 56/875 performed tests (6%) from 6/82 patients (7%). Tests were repeatedly positive for the same organism in sequential samples from each of the 6 patients, identifying coagulase-negative staphylococci (n=3), enterococci (n=2) and P. aeruginosa (n=1). 5 of 6 patients developed infection (3 sepsis, 2 without focus) during hospitalisation. DNA of the respective organism was detected in blood by SeptiFast 2–23 d (median 10 d) before the same organism was detected by blood cultures collected during the febrile episode. In all 6 patients, DNAemia sequentially increased until start of adequate antibiotic therapy.

**Conclusions**: In haematological patients, SeptiFast detected DNAemia 2–23 d before occurrence of the febrile episode and positive blood cultures. An automated molecular diagnostic test may be useful for screening blood of neutropenic patients, allowing an intervention before occurrence of fever.

**P1763** How diverse can be the management of this special group of at-risk patients? A regional multidisciplinary audit on febrile neutropenia in hospitals of northwestern England

A. Galeri, I. Thakur, R. Sharma* (Blackpool, UK)

**Background**: Febrile neutropenia [FN] is a common emergency in Haemat-m-oncology. Optimal management FN is crucial to improving outcomes for patients with potentially curable malignancies. However, currently there are no national guidelines on diagnosis and management of FN. There is an apparent inter-hospital variation in FN policies and in currently available international and regional guidance documents. Blackpool Victoria Hospital is a large district hospital [DGH] offering enhanced regional haematology services. A regional audit was carried out jointly by Clinical Microbiology and haematology.

**Methods**: Completed questionnaires from 14 hospitals in the region were analysed. Details of audit to be presented. Standards on FN included guidance documents from IDSa, BCsh, Christie hospital [CH] and www.uptodate.com

**Results**: Respondents included DGH [10/14]; Teaching hospitals [2/14] and Specialist tertiary [2/14]. Microbiologist and haematologist jointly co-author FN policies. Over 90% policies address initial clinical assessment, investigations and first line antibiotics; 50% mention risk stratification [without any reflection in the policy]; over 71% discuss subsequent assessment and treatment modification; 42% made reference to any guideline documents; Variations [14–28%] in definitions of fever and neutropenia was noted. Antifungal use follows clinical suspicion [85%]; BAL culture and HRCT [78%]; Galactomannan EIA [-10%]. 85% consider antifungal on day 4/5 unresponsive fever while 15% at 72hs. Lisosomal amphotericin and caspofungin were common options. Itraconazole [prophylaxis] in leukaemia/lymphoma patients [-90]. Piperacillin-tazobactam/gentamicin or carbapenem used 1st line

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Figure: Definition of FEVER in the policy.

**References**: www.uptodate.com

**Results**: Respondents included DGH [10/14]; Teaching hospitals [2/14] and Specialist tertiary [2/14]. Microbiologist and haematologist jointly co-author FN policies. Over 90% policies address initial clinical assessment, investigations and first line antibiotics; 50% mention risk stratification [without any reflection in the policy]; over 71% discuss subsequent assessment and treatment modification; 42% made reference to any guideline documents; Variations [14–28%] in definitions of fever and neutropenia was noted. Antifungal use follows clinical suspicion [85%]; BAL culture and HRCT [78%]; Galactomannan EIA [-10%]. 85% consider antifungal on day 4/5 unresponsive fever while 15% at 72hs. Lisosomal amphotericin and caspofungin were common options. Itraconazole [prophylaxis] in leukaemia/lymphoma patients [-90]. Piperacillin-tazobactam/gentamicin or carbapenem used 1st line

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Figure: Definition of FEVER in the policy.
Empirical use of teicoplanin versus vancomycin in febrile neutropenic patients at high risk for Gram-positive bacteraemia: results of a multi-centre prospective randomised clinical trial


Objectives: To evaluate the efficacy and safety of empirical use of teicoplanin versus vancomycin in febrile neutropenic patients at high risk of Gram positive bacteraemia.

Methods: A total of 190 febrile neutropenic patients from 21 centres were randomised to receive teicoplanin (97 patients) or vancomycin (93 patients) in addition to a standard empiric regimen of intravenous cefazidime and amikacin for duration of 5 to 21 days depending on response to the treatment.

Results: There was no difference between teicoplanin and vancomycin groups in terms of overall survival rates (92.5% vs. 92.6%, p > 0.05) and response rates (55.7% vs. 53.3%, p > 0.05). Gram positive isolates from peripheral blood cultures were more than Gram negative isolates (62.2%). Similar adverse events without any statistical significance occurred in both groups; most common ones being hypokalaemia, rash, diarrhea, and hepatotoxicity. There was no difference between treatment groups according to adverse events.

Conclusion: Teicoplanin is as effective as vancomycin in terms of treatment response and overall survival rate in febrile neutropenic patients. Both antibiotics have acceptable safety profiles.

Three-day treatment with imipenem for unexplained fever during prolonged neutropenia. A prospective observational study on the safety in haematology patients receiving fluoroquinolone and fluconazole prophylaxis

L. Slobbe*, B. Rijnders (Rotterdam, NL)

Objectives: Guidelines advocate at least 7 days of broad-spectrum antimicrobial therapy for unexplained fever during neutropenia. However, effective antimicrobial prophylaxis reduces the incidence of Gram-negative infections, which may allow shorter treatment. This study evaluates the safety of discontinuing empirical broad-spectrum antibiotics if no microbial source is documented after 72 hours.

Methods: Prospective observational study at a tertiary care haematology unit in patients suffering from haematologic malignancies, with treatment induced prolonged neutropenia of ≥10 days. Oral fluoroquinolone and fluconazole prophylaxis was given from day 1. A standardised diagnostic protocol was followed and fever was empirically treated with imipenem, which was discontinued after 72 hours if no infectious aetiology was documented. Duration of fever, antimicrobial therapy and all-cause mortality 30 days after neutrophil recovery were registered.

Results: 166 patients were evaluated during 276 neutropenic episodes. 29 patients (17.5%) did not develop fever during any episode. 137 patients (82.5%) experienced ≥1 febrile episodes. A total of 317 febrile episodes were observed, of which 177 (56%) were diagnosed as unexplained fever (UF). In 135 febrile episodes (43%), a probable/definite infectious origin was documented. Mean duration of fever in neutropenic periods with 1 febrile episode was 5 days, with imipenem given for 4.7 days. In patients with UF, imipenem was given for 3.7 days. All-cause mortality 30 days after neutrophil recovery was 3.6% (6/166); in 4 of these patients, an infectious origin could not be established.

Conclusion: Discontinuation of broad-spectrum antimicrobial therapy given for unexplained fever during neutropenia in haematology patients on fluoroquinolone and fluconazole prophylaxis is safe if no infectious origin is found after 72 hours.

Antiviral prophylaxis in haematological patients: systematic review and meta-analysis

D. Yahav, A. Gafter-Gvili, E. Muchtar, K. Skalsky, G. Karic, M. Yeshurun, L. Leibovici, M. Paul* (Petah Tikva, IL)

Objectives: Herpesviruses cause major morbidity among haematological patients undergoing haematopoietic stem cell transplantation (HSCT) or following chemotherapy. We conducted a systematic review and meta-analysis to quantify overall patients’ gain with antiviral prophylaxis in specific clinical scenarios.

Methods: Included were randomised controlled trials assessing antiviral prophylaxis vs. placebo, no treatment, preemptive treatment or another antiviral drug. Patients undergoing HSCT or intensive chemotherapy for acute leukaemia or high-grade lymphoma were included. No restrictions on language, year or publication status were applied. All-cause mortality, herpes simplex HSV and cytomegalovirus (CMV) disease were assessed as primary outcomes. Pooled relative risks (RR) and numbers needed to treat (NNT) with 95% confidence intervals are reported.

Results: HSCT was the condition assessed in 22 trials and intensive chemotherapy in 5 trials. In the pre-engraftment setting of autologous or allogeneic HSCT, antiviral prophylaxis (mainly acyclovir for HSV seropositive recipients or donors) significantly reduced HSV (NNT 2,
2–2) and CMV disease, with no effect on all-cause mortality. In the allogeneic post-engraftment setting (mainly CMV-seropositive recipients/donors), antiviral prophylaxis resulted in a significant reduction in all-cause mortality, RR 0.79 (0.65–0.95), NNT 12 (7–50) and all viral-related outcomes. The effect on CMV was more pronounced with ganciclovir (5 trials) and maribavir (1 trial), but acyclovir alone (7 trials) also significantly lowered mortality. During chemotherapy, acyclovir significantly decreased HSV disease (NNT 3, 2–4) and infection rates, with no effect on mortality. HSV disease represented mostly HSV-positive oral mucositis. Overall mucositis and pneumonitis rates were not reported. Small study’s effect was observed for viral-related outcomes.

Conclusions: Antiviral prophylaxis reduced mortality with a small NNT in the post-engraftment setting of allogeneic HSCT and should be administered to all CMV-seropositive HSCT recipients. Since prophylaxis in this setting significantly reduced VZV and HSV disease rates, consideration should be given to the use of prophylaxis also for VZV-seropositive or HSV-seronegative (CMV seronegative) recipients. During the pre-engraftment period and for patients undergoing intensive chemotherapy, antiviral prophylaxis does not reduce mortality and its effect on overall patient morbidity is unknown.

**P1767** Cytomegalovirus disease among immunocompromised non-transplanted patients

M. Chitasombat, S. Watcharanunan*, V. Chantrititaya, S. Sungkamparporn (Bangkok, TH)

Objectives: To study clinical characteristics, outcome and predicting factors of death among immunocompromised non-transplanted patients who had Cytomegalovirus (CMV) disease

Methods: A retrospective study was conducted among non-transplanted patients who received immunosuppressive therapy and were diagnosed with CMV disease between January 2005 and December 2008.

Results: CMV disease occurred in 34 patients, 21 (61.7%) and 13 (38%) of whom had definite and probable diagnosis, respectively. Median (IQR) age of patients was 49 (40–60) years. Systemic lupus erythematosus (SLE) was the major underlying disease, noted in 21 (61%) patients. Major immunosuppressive agents included prednisolone (34, 100%) and oral endoxan (16, 47%). Pulse methyl prednisolone, pulse endoxan, mycophenolate mofetil and rituximab was used in 21 (61.8%), 15 (44%), 2 (5.8%) and 2 (5.8%) patients, respectively. Among CMV disease, pneumonitis was the most common (26, 75.6%), followed by enterocolitis (10, 29.4%), and disseminated infection (6, 17.6%). Overall, the median (IQR) blood CMV viral load (VL) was 12200 (1820–55125) copies/mL. Among patients with disseminated CMV disease was 43,450 (1497–73,075) copies/mL. Other opportunistic infections were noted in 23 (67.7%). Of these, pulmonary aspergillosis was the most common (16 patients, 47.1%), followed by candidiasis (11, 32.4%), and PCP (10, 29.4%), respectively. Active tuberculosis was noted in 5 patients (14.7%). The overall mortality rate was 67.6%. Major causes of death were respiratory failure (26.6%) and sepsis (26.6%). From multivariate analysis, predicting factors of death were recent use of intravenous pulse endoxan (p = 0.001), use of mycophenolate mofetil or rituximab (p = 0.031), blood CMV viral load (p = 0.029), co-infection with PCP (p = 0.008), pulmonary aspergillosis (p = 0.02) and active tuberculosis (p = 0.007).

Conclusion: CMV disease is a significant complication resulting in a high mortality among immunocompromised non-transplanted patients. Our study reflected the advanced course of CMV disease among this severely immunosuppressed population. Blood monitoring of CMV viral load is suggested for early detection of CMV infection among immunocompromised non-transplanted patients.

**P1768** Detection of cytomegalovirus resistance to antivirals in paediatric haematopoietic stem cell transplant recipients: study in a paediatric cohort in the Czech Republic


Objectives: Despite the improvement of infection monitoring and antiviral treatments, cytomegalovirus (CMV) infections remain a major cause of morbidity and mortality in allogeneic haematopoietic stem cell transplant (alloH SCT) recipients. CMV resistance to antivirals, which is one reason for treatment failure, was investigated in a paediatric population.

Methods: Between 2002 and 2008, 6339 whole blood samples (median: 30/patient) from 192 alloH SCT patients (median age at HSCT: 8.9 yrs) were tested. After DNA extraction, CMV genome and albumin gene were quantified using real-time quantitative PCR, and results were expressed as normalised viral copies (NVCs) per 100000 human genome equivalents. First-line antiviral treatment, usually ganciclovir (GCV), was initiated when CMV load exceeded 1000 NVCs, and switched to fosarnet or cidofovir in case of none response or clinical signs of CMV infection. If clinical resistance was suspected, based on the absence of viral response after 2 weeks of a well-conducted treatment, CMV resistance was studied by restriction analysis and sequencing of UL97 phosphotransferase and UL54 DNA polymerase genes.

Results: CMV load was over 100 NVCs in 839 samples from 86 patients (45%), over 1000 NVCs in 346 samples from 55 patients (29%) and 10000 NVCs in 87 samples from 20 patients (10%). Despite treatments, mild signs of CMV infection developed in 22 patients (11%), and CMV disease was observed in 8 patients (4%; 6 pneumonias, encephalitis and colitis). Seven patients died in consequence of CMV infection. Among the 22 patients (11%) with suspicion of resistance, genotypic resistance of CMV was evidenced in 4 patients (7%), with median of 273 days post-alloHSCT and CMV load of 10277 NVCs. Resistance to GCV was associated with mutations L595S and A591V in UL97, del981–982, N408K, V715M and P522S in UL54. Natural chemotherapies were administered to all CMV-seropositive HSCT recipients. Since expression as normalised viral copies (NVCs) per 100000 human genome equivalents. First-line antiviral treatment, usually ganciclovir (GCV), was initiated when CMV load exceeded 1000 NVCs, and switched to fosarnet or cidofovir in case of none response or clinical signs of CMV infection. If clinical resistance was suspected, based on the absence of viral response after 2 weeks of a well-conducted treatment, CMV resistance was studied by restriction analysis and sequencing of UL97 phosphotransferase and UL54 DNA polymerase genes.

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Conclusions: CMV load normalisation is useful for the surveillance and the treatment of CMV infection after alloHSCT. Genotypic testing of CMV resistance proved to be important in case of clinical resistance. Further studies are required to ascertain the true nature of the novel mutations within UL97 and UL54 genes appearing after prolonged antiviral treatments. Supported by VZ FNMI42003, MSTM001260813, NR9418–3

**P1769** Treatment failure and antiviral resistance in cytomegalovirus infections in stem cell transplant recipients

M.T. van der Beek*, E. Marritt, A.C.T.M. Vossen, C.S. van der Bijl-de Brouwer, C.J.M. Haakse, E.C.J. Claas, A.C.M. Kroes (Leiden, NL)

Objectives: Treatment of cytomegalovirus infections after stem cell transplantation does not always lead to a rapid and sustained viral response. It is important to unravel possible causes of treatment failure to improve therapeutic strategies. This study investigated the occurrence and risk factors of treatment failure in CMV infections in SCT patients, including the role of antiviral resistance.

Methods: Ninety-two consecutive adult recipients of allogeneic T-cell depleted stem cell transplants who were at risk for CMV (donor and/or recipient CMV seropositive) were studied retrospectively. CMV infections had been treated with (val)ganciclovir according to a preemptive strategy, based upon regular monitoring of the CMV-DNA load in plasma. Patient charts were reviewed for patient and transplantation characteristics and antiviral treatment data. Treatment failure was defined
as a CMV DNA load of at least 1000 copies/ml after at least 2 weeks of treatment. Resistance was analyzed by nucleotide sequence analysis of the UL97 and UL54 genes in the first CMV DNA positive sample of all patients and in follow-up samples during treatment failure.

Results: Pre-emptive treatment for CMV infections was administered in 51 of 92 patients; 24% of seronegative recipients and 63% of seropositive recipients, irrespective of donor serostatus (Pearson Chi-Square, p < 0.05).

Treatment failure occurred in 49% of the treated patients (25 of 51).

A high maximum CMV DNA load correlated with treatment failure ( Spearman correlation coefficient, p < 0.01); treatment failure was found in 27%, 63% and 100% of patients with a maximum CMV load of 10 log 4, 10 log 5 and at least 10^log 6 copies/ml respectively. No clear association was found between CMV serostatus of donor or recipient, donor type (related/unrelated) or conditioning regimen (myeloablative/ non-myeloablative) and the risk of treatment failure.

In 1 patient, who developed CMV encephalitis during treatment failure, ganciclovir-resistant viral isolates with the well-characterised AS94V mutation in the UL97 gene were found.

Conclusion: In stem cell transplant patients, CMV infections with a slow response to antiviral treatment occur frequently. Antiviral resistance was observed but apparently played a minor role in treatment failure.

Clinical applicability of a diagnostic DNA-microarray for the simultaneous detection of herpesviruses and adenovirus co-infections in patients undergoing allogeneic stem cell transplantation


Introduction: Herpesviruses and adenovirus are an important cause of end-organ disease (EOD) and are associated with graft versus host disease (GVHD) in patients undergoing allogeneic stem cell transplantation (SCT). Since more than one herpes- or adenovirus are reported to be present shortly after transplantation, we validated a DNA-microarray (VINAry) for the simultaneous detection and monitoring of those viruses. Still, evaluations as to the best possible clinical and diagnostic applicability of the array have not been performed.

Methods: We studied the (simultaneous) viral reactivation of herpessimplex-virus-1 and -2 (HSV-1/2), cytomegalovirus (CMV), varicella-zoster-virus (VZV), Epstein-Barr-virus (EBV), human herpesvirus-6 (HHV-6) and adenovirus of 35 patients during the first 100 days after allogeneic SCT. Time post SCT was devised in phase 1 (pre-engraftment, day 0–14), phase 2 (engraftment, day 15–30), phase 3 (post-engraftment, day 31–60) and phase 4 (late phase, >day 60). We evaluated the frequency and diversity of viral (co-)reactivation in the different phases post SCT.

Results: In phase 1, samples of 34 patients (97.1%) were available, in phase 2 and 3 of 32 patients (91.4%) and in phase 4 of 29 patients (82.9%), respectively. In the different phases, 17 of 34 patients (50%) had positive samples in phase 1, 19 of 32 patients (59%) in phase 2, 27 of 32 patients (84%) in phase 3 and 24 of 29 patients (72%) in phase 4. Among the positive patients, CMV was detected most frequently (58% in phase 1, 57% in phase 2, 88% in phase 3, 83% in phase 4), followed by HHV-6 (33%, 57%, 41%, 33%), EBV (12%, 26%, 38%, 33%) and adenovirus (12%, 22%, 19%, 20%), respectively. Simultaneous infections with 2 viruses were diagnosed in 12% of positive patients in phase 1, 36% in phase 2, 19% in phase 3 and 33% in phase 4. Triple infections were diagnosed in 0%, 5%, 14% and 8% of positive patients, respectively.

Conclusion: We conclude that monitoring of multiple viral infections is necessary in patients undergoing SCT, since clinical symptoms of viral reactivation of herpes- and adenovirus may be very similar. Best diagnostic applicability seems to be in phases of engraftment and post-engraftment. By screening and monitoring SCT-patients for viruses known to be associated with EOD or GVHD we herewith introduce an innovative molecular technique that can simultaneously detect multiple viral infections on a large scale.

Incidence, timing and aetiology of bloodstream infections following orthotopic liver transplantation or haematopoietic stem cell transplantation – a single-centre experience


Bloodstream infections (BSI) are major complications of orthotopic liver transplantation (OLT) and haematopoietic stem cell transplantation (HSCT). Knowing characteristic timing of their occurrence enables anticipation of these infections and their earlier detection, and knowing the pattern of causative microorganisms is a prerequisite for determining suitable empirical therapy.

Objective: to evaluate and compare incidence, timing and aetiology of BSI post transplantation (TX) in two groups (OLT and HSCT) of patients in a single institution.

Methods: 263 consecutive TXs performed from Jan 2005 to Oct 2008 have been evaluated. OLT patients: n = 136; mean age 49 (range 16–75, SD 12), HSCT patients: n = 126; mean age 45 (range 19–70, SD 14); autologous TX 80.2%, allogeneic 19.8%. Patients were followed up 1 year after TX; blood culture dates and isolates were recorded.

Results: 78 BSI were identified (OLT: n = 42, HSCT: n = 36) at a median of 18 (range 1–256, SD 75) days post TX (OLT: median 42, range 1–248 SD 65 days; HSCT: median 8, range 1–256, SD 85 days, Mann Whitney p = 0.004). In both groups, the majority of BSI were observed in the first trimester following TX, in HSCT patients mostly (69.4% of all BSI in HSCT) during the first two weeks (Fig. 1). Gram-negative (GN) organisms were the prevalent cause (52.6%) of BSI in both groups, with Pseudomonas aeruginosa accounting for 16.7% of all BSI and 31.7% of all GN BSI. Gram-positive (GP) pathogens were responsible for 37.2% of BSI, with coagulase-negative staphylococci being the most prevalent in this group (17.9% of all pathogens. 48.3% of all GP organisms). Mixed BSI represented 3.8% and fungaemias (candidaemias) 6.4% of all BSI. No statistically significant differences in aetiology of BSI (GN, GP, fungal) were found between OLT and HSCT patients, neither during the whole period of observation (1 year), nor in any of the 4 trimesters. A trend towards higher incidence of fungaemia in OLT and allogeneic HSCT compared to autologous HSCT patients was revealed (Chi square, p = 0.09).

Conclusions: BSI are frequent complications of both OLT and HSCT, especially during the first trimester post TX. In HSCT patients they occur significantly earlier than in OLT patients. Significant differences in aetiology of BSI between OLT and HSCT patients were not found, most BSI were caused by GN organisms in both groups. Empirical therapy in these immunosuppressed patients should include agents with strong antipseudomonal activity.

Figure 1. Incidence of bloodstream infections in patients after haematopoietic stem cell transplantation (HSCT) or orthotopic liver transplantation (OLT).
Outbreak of *Ralstonia pickettii* bacteremia in patients with haematological malignancies and haematopoietic stem cell transplant recipients

M. Mikalska*, M. Alberti, P. Durando, M.P. Molinari, M.T. Van Lint, S. Bregante, A. Dominietto, A.M. Raitola, V. Del Bono, G. Icardi, G. Orenzo, A. Bacigalupo, C. Viscoli (Genoa, IT)

**Objectives:** *Ralstonia pickettii* is a non-fermenting Gram-negative rod commonly found in soil and moist environments. It is rarely isolated from clinical specimens or associated with infections, although blood stream infections (BSI) have been reported.

**Methods:** We describe a series of 11 *R. pickettii* BSI occurring over a period of 3 months (3/06/08–19/08/08) in 10 patients with haematological malignancies or after haematopoietic stem cell transplant (HSCT). All the patients werereviewed and actively monitored. All the cultures resulted negative. Additionally, all the existing hygiene and infection control procedures and environmental samples, together with samples of several potential pathogens, were reviewed. Epidemiological and microbiological investigations were undertaken.

**Results:** Clinical and microbiological features of the patients are shown in Table 1.

<table>
<thead>
<tr>
<th>Dates</th>
<th>Age</th>
<th>Gender</th>
<th>Underlying disease</th>
<th>Date of HSCT</th>
<th>Date of first symptoms</th>
<th>Total blood cultures</th>
<th>CVC</th>
<th>Source of blood culture</th>
<th>Source to blood culture negative</th>
<th>Note</th>
</tr>
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<tr>
<td>1.</td>
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<td>M</td>
<td>55 NHL</td>
<td>03/06/08</td>
<td>05/06/08</td>
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<tr>
<td>2.</td>
<td>DO</td>
<td>M</td>
<td>24 AML</td>
<td>17/06/08</td>
<td>19/06/08</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>HD</td>
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</tr>
<tr>
<td>3.</td>
<td>SL</td>
<td>M</td>
<td>40 ALL</td>
<td>26/06/08</td>
<td>26/06/08</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>51</td>
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<tr>
<td>4.</td>
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<td>F</td>
<td>59 NHL</td>
<td>23/07/08</td>
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<td>1</td>
<td>1</td>
<td>Neg</td>
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<tr>
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<td>M</td>
<td>54 AML</td>
<td>23/07/08</td>
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<td>1</td>
<td>Neg</td>
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<tr>
<td>6.</td>
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<td>M</td>
<td>36 ALL</td>
<td>21/08/08</td>
<td>06/08/08</td>
<td>6</td>
<td>4</td>
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<td>51</td>
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<td>M</td>
<td>63 MF</td>
<td>24/01/08</td>
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<td>5</td>
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*Two separate episodes of *R. pickettii* bacteremia, the first one occurred on 16th June 2008, and was treated in a different hospital.

We describe a series of 11 *R. pickettii* BSI occurring over a period of 3 months (3/06/08–19/08/08) in 10 patients with haematological malignancies or after haematopoietic stem cell transplant (HSCT). All the patients were reviewed and actively monitored. All the cultures resulted negative. Additionally, all the existing hygiene and infection control procedures and environmental samples, together with samples of several potential pathogens, were reviewed. Epidemiological and microbiological investigations were undertaken.

**Results:** Clinical and microbiological features of the patients are shown in Table 1.

32 isolates were recovered from blood and 1 from a catheter tip. All patients had a central venous catheter at the time of BSI. The isolates were susceptible to amoxicillin, cefuroximemol, 3rd and 4th generation cephalosporins, piperacillin/tazobactam and carbapenems, and resistant to aminoglycosides.

In 5/11 BSI, the patients had a full-blown sepsis syndrome, while the other 6 episodes were free of symptoms, with no increase in C-reactive protein. All the patients received intravenous antibiotic therapy with 4th generation cephalosporins, piperacillin/tazobactam and carbapenems. All the patients received intravenous antibiotic therapy with 4th generation cephalosporins, piperacillin/tazobactam and carbapenems. All the patients received intravenous antibiotic therapy with 4th generation cephalosporins, piperacillin/tazobactam and carbapenems.

**Conclusion:** We report successful treatment and control of an outbreak of *R. pickettii* BSI in a HSCT Unit. Although *R. pickettii* is a pathogen with low intrinsic virulence and it might be a contaminant of blood cultures, it should not be overlooked when it is repeatedly recovered from sterile body fluids, especially in immunocompromised hosts who lack both classical signs and symptoms of the sepsis and full capacity to fight infections.

**Tailor-made therapy to prevent postoperative sepsis in living donor liver transplantation**

T. Kaido*, H. Egawa, S. Uemoto (Kyoto, JP)

**Objectives:** Recipients undergoing liver transplantation (LT) have an extremely high risk of postoperative infection leading to lethal sepsis from various aetiologies including preoperative impaired immunological competence due to protein energy malnutrition, major surgery, and postoperative immunosuppressive treatment. Therefore, the results of LT can be improved by prevention of postoperative severe infection. We describe our tailor-made preventive therapy against postoperative sepsis in living donor LT (LDLT).

**Methods and Results:** 1) Cause of in-hospital death: We retrospectively analyzed 1282 consecutive cases that underwent LDLT at a single medical centre between June 1990 and December 2007. The most frequent cause of in-hospital death was sepsis (34%) followed by multiple organ failure (20%), and primary graft nonfunction or acute cellular rejection (12%) in numerical order. This finding demonstrates that postoperative sepsis is central to improvement in short-term outcome of recipients.

2) Preoperative immunosuppressive treatment: We reported that in liver transplant recipients undergoing LT, CD8+ T-cell subpopulation enriched with cytotoxic T lymphocytes was associated with a significantly higher rate of infection and a lower survival rate after LT. Based on these results, we have modified the immunosuppressive treatment for this subpopulation in order to prevent postoperative infection since January 2007. We reduced the daily dose of steroids in ABO-incompatible patients and stopped postoperative steroid administration in ABO-identical or -compatible patients. The incidence of sepsis as well as in-hospital mortality was significantly decreased, especially in ABO incompatible patients (p < 0.001).

3) Preoperative nutritional assessment: In February 2008, we introduced a new preoperative nutritional assessment using a body composition analyzer on admission. Patients showing poor nutrition preoperatively had a significantly higher incidence of postoperative sepsis than well-nourished patients. Therefore, we have started aggressive preoperative nutritional intervention for poorly nourished patients including supplementation of branched-chain amino acid-enriched nutrient mixture and got good results.

**Conclusion:** Preoperative immunological status and nutritional assessment could predict a high-risk group for postoperative sepsis. Tailor-made therapy to prevent postoperative sepsis in high-risk patients would be useful to improve the results of LDLT.

**Widespread subcutaneous nodules as a manifestation of breakthrough invasive aspergillosis in a bone marrow transplant patient: assessment of disease extension by positron emission tomography scan imaging**


Invasive aspergillosis often presents as a respiratory tract infection in transplant patients, but after haematogenous dissemination virtually any other organ may be involved. Metastatic cutaneous aspergillosis usually presents as necrotizing cutaneous papules or ulcerating skin lesions due to embolisation and skin infarction. We report an unusual form of disseminated aspergillosis and the usefulness of positron emission tomography (PET) scan imaging in assessing its extension. An acute leukaemia patient underwent an unrelated mismatched non-myeloablative allogeneic stem cell transplantation after highly immunosuppressive conditioning. Primary antifungal prophylaxis was with itraconazol oral solution. An episode of acute gut graft vs host disease was treated with high dose steroids plus extra-corporeal photopheresis and itraconazol was replaced by oral voriconazol. He
A decision support system for diagnosis and treatment of gastrointestinal tract infections in solid-organ transplant recipients

E. Goldberg*, G. Kariv, V. Shani, J. Bishara, L. Leibovici, M. Paul for the TREAT Study Group

Objectives: To build a computerised decision support system for diagnosis and treatment of suspected gastrointestinal tract infections in solid organ transplant patients, as a part of a larger causal probabilistic network (CPN) that will include other sites of infection (TREAT).

Methods: The information used to build the model was obtained from existing local databases of infections and antimicrobial resistance patterns, and systematic literature searches. We used the HUGIN graphical interface to build a CPN model and populated it with the incidences and probabilities obtained from the search. We prospectively identified solid organ transplant patients, hospitalised due to diarrhoeal disease (passage of three or more abnormally liquid or unformed stools for at least two days) during the years 2007–2008 in our hospital. We compared the model’s prediction for each patient with the empirical diagnosis given by the managing clinicians. The gold standard for comparison was the final microbiological diagnosis. This analysis was meant to determine the safety of the model before it is joined with the larger network.

Results: The CPN model includes the different pathogens that may cause diarrhoea in solid organ transplant patients, specific risk factors for each pathogen, anti-rejection drugs that may induce diarrhoea, clinical symptoms and laboratory tests. Twenty solid organ transplant recipients presenting primarily with gastrointestinal manifestations were included. Mean age was 53 years (range 29–75). Most patients were men (17/20), and most underwent kidney transplantation (15/20).

Our model predicted the correct diagnosis in 14/20 cases (70%), compared with 4/20 (20%) correct initial diagnoses of the treating physicians. The model predicted the presence of CMV infection in 8 cases, of which 5 were microbiologically documented. One case of diagnosed CMV was not predicted by the model. ROC analysis for CMV infection yielded an area under the ROC curve of 0.75 (95% CI 0.501–0.999).

Conclusions: We could create a safe and reliable model to support clinicians in diagnosis of infectious causes of diarrhoea in solid organ transplant patients. Using this model may shorten the time to diagnosis, prevent unnecessary antibiotic treatment and prevent future resistance. This model will be incorporated into a larger network dealing with all sites of infection in these patients.

Amphotericin B as antifungal prophylaxis in liver transplantation

A. Talento*, S. McDermott, A. Gilleece, A. McCormick, L. Fenelon, K. Schaffer (Dublin, IE)

Objective: The rate of fungal infections in orthotopic liver transplant (OLT) patients ranges from 5 to 42%. Antifungal prophylaxis has been shown to decrease the incidence of fungal infections. In this study, we wanted to analyze the efficacy of liposomal/lipid complex amphotericin B as universal antifungal prophylaxis in OLT patients.

Methods: We conducted a retrospective chart review of OLT patients who were treated for fungal infections over a 15 year time period from January 1993 to December 2007. Two separate time periods were analyzed: 1. From 1993 to 1999, 71% of patients received fluconazole, 10% liposomal amphotericin B, 15% oral amphotericin B and 3% received both fluconazole and amphotericin B as antifungal prophylaxis; 2. From 2000 to 2007, liposomal or lipid complex amphotericin B was given for universal antifungal prophylaxis due to building works that commenced in the hospital early in 2000. Only patients who did not tolerate liposomal amphotericin B were given lipid complex amphotericin B as prophylaxis(10%).

Results: There were a total of 506 OLTs performed on 463 patients during the 15 year period. One hundred thirty six OLTs were performed in 117 patients during the first period while there were 370 liver transplants in 346 patients in the second period. During the first period, 21 patients out of 117(18%) were treated for fungal infections compared to 21 patients out of 346 (6.1%) during the second period. This is a significant decrease in fungal infections between the two time periods (chi square 15.03, p value <0.001).

Conclusion: In our centre, universal prophylaxis with liposomal/lipid complex amphotericin B was found to be efficient in preventing fungal infections. Other factors that most likely contributed to the observed decrease are improved surgical techniques and new, more specific immunosuppressive agents.
Long-term evaluation of preemptive treatment after solid organ transplant in patients at high risk for cytomegalovirus infection

O.J. Ben Marzouk-Hidalgo, E. Cordero, A. Martín-Peña, E. García-Prado, B. Sánchez, M.A. Gómez-Bravo, M.A. Gentil, J.M. Cisneros, P. Perez-Romero (Seville, ES)

**Objectives:** To determine if in solid organ transplant (SOT) recipients at high risk for cytomegalovirus (CMV) infection, pre-emptive valganciclovir (VGC) therapy guided by a sensitive diagnostic method prevents CMV disease and allows early activation of a CMV specific immune response.

**Methods:** SOT patients, seronegative for CMV receiving a seropositive graft, were enrolled. Viral load (VL) was determined periodically, from week 2 post-transplantation for a total of 18 months by real-time PCR (rt-PCR), which was used to guide VGC administration when VL was over 1,000 cop/ml. Twin samples were used to detect infection by antigenemia. The CMV-specific T-cell immune response was measured by flow cytometry using specific cell surface markers (CD69, CD3, CD4 and CD8) and cytokine production (IL-4 and IFN-γ). In addition, the emergence of resistance mutations in UL97 and UL54 was determined, and treatment adherence was characterised by measuring plasma VGC levels using HPLC.

**Results:** Ten patients fulfilled the study requirements and a total of 230 plasma samples were collected. In 42.6% of the samples the results were discordant for CMV infection, with rt-PCR more sensitive than antigenemia. Moreover, within the same patients positive antigenemia was delayed by two to three weeks compared to positive rt-PCR. The highest risk for infection after the transplant occurred between days 43 and 63. Treated episodes over 1,000 copies/ml occurred between days 28 and 119. No disease symptoms or graft rejection related to CMV infection were detected. Nine patients acquired a specific immune response against CMV between days 84 and 98 post-transplantation. One patient acquired immunity at day 140, which correlated with a lower VL. After acquisition of immunity, nine patients cleared new CMV episodes without VGC administration. One patient did not control the infection, which correlated with the emergence of the M460 mutation in UL97, and suboptimal levels of VGC in plasma.

**Conclusion:** Pre-emptive therapy guided by rt-PCR can be used successfully for SOT patient at high risk to prevent CMV disease. In addition, pre-emptive therapy allows for an interaction between viral antigens and host immune system, which results in a specific immune response against CMV that further controls infection without treatment administration. A lack of control of infection after the immunity was associated with the emergence of viral resistance due to poor treatment adherence.

Current status of CMV reactivations in adult liver transplant patients monitored by frequent quantitative PCR testing

I. Lautenschlager*, R. Lognec, H. Mäkisalo, K. Höckerstedt (Helsinki, FI)

**Objectives:** Cytomegalovirus (CMV) is a significant infectious agent causing morbidity in transplant patients. CMV-infection mostly appears within 2–3 first months after transplantation. To prevent CMV, most liver centres use prophylaxis for high risk patients of CMV-seronegative recipients receiving an organ from a seropositive donor (R–D+) and many centres even for all seropositive recipients (R+). Preemptive treatment is mainly used for those at a moderate or low risk of CMV, with a major advantage of reduced drug expose. Preemptive therapy is based on the screening for early evidence of CMV by frequent monitoring of viral load. The current status of CMV-reactivations demonstrated by quantitative PCR-monitoring of adult CMV-seropositive (R+) liver transplant patients was studied.

**Patients and Methods:** Altogether 211 adult patients were transplanted 2003–2007. The basic immunosuppression consisted of CNI inhibitors, azathioprine/MMF plus steroids. High risk patients received valganciclovir (or ganciclovir) prophylaxis, i.v. ganciclovir was used for preemptive therapy for (R+) patients, and in the case of symptomatic CMV. Most recipients, 176 (84%), were CMV-seropositive (R+). The patients were frequently monitored for CMV by a TaqMan based real-time quantitative plasma PCR, which correlates with the commercial quantitative CMV-PCR Cobas Amplicor Monitor. Of those, 161 (R+) patients with a follow-up over six months were studied.

**Results:** In most cases, 98/161 (61%) no evidence of CMV was seen, and just 63/161 (39%) developed CMV-DNAmiesia during the post transplant monitoring of six months. Only 25/63 reactivations exceeded 5000 copies/ml considered as cutoff level for preemptive treatment (median 21500, range 5100–813300 copies/ml), and most had self-limiting, low-level CMV-DNAemia (median 850, range 234–4000 copies/ml). Thus, low-level temporal CMV-reactivation occurred in 38/161 (R+) patients (23.5%), and only 25/161 (15.5%) demonstrated significant viral loads. No correlation to immunosuppression regimen could be found. No patient or graft was lost due to CMV.

**Conclusion:** These results demonstrate that most CMV-seropositive adult liver recipients do not develop CMV-reactivation, and even if reactivations occur, most of them are temporal, low-level DNAemias. Thus, universal prophylaxis for all R+ patients would not seem to be reasonable in this patient population.

Valganciclovir prophylaxis for CMV infection in thoracic transplant patients


Cytomegalovirus (CMV) infection is a very common opportunistic infection after solid organ transplantation. Intravenous Valganclovir (vGCV), an haematotoxict drug with renal elimination pathway, remains the first-line treatment for CMV disease. Due to its poor bioavailability, oral GCV was replaced by an oral prodrug valganciclovir (vGCV) for prophylaxis.

**Objectives:** We analyzed In a retrospective study, the efficacy and safety of vGCV during and for 4 months after discontinuation of prophylaxis in heart (HT) and lung transplant patients with (CFLT) cystic fibrosis or not (LT).

**Methods:** Selected patients were HT, LT and CFLT, with a stable renal function (RF) receiving 900 mg vGCV daily for preventing CMV disease between 2005 and 2007. Prophylaxis was introduced in the early post transplantation period during respectively 3 to 6 months in HT and to 12 months in CFLT and LT. Donor (D) and recipient (R) CMV serostatus were collected. A GCV therapeutic drug monitoring (TDM) was realised to document efficient concentrations in the 0.5–1.5 mg/L range. Plasma GCV trough levels were measured by UV-LC assay. Moreover, efficacy was checked by pp65 antigenemia (Ag) detection in peripheral blood leukocytes.

**Results:** 32 thoracic transplants (11 HT, 7 LT, 14 CFLT) were included into the study. CMV serostatus distribution was 53% D+/R-, 25% D–/R+ and 22% D–/R–. vGCV was maintained for 106±6.7 days in case of HT versus 270±8.5 days for LT and CFLT. 300 determinations of GCV through concentrations have been performed, representing 7 to 12 samples per patient. HT, LT and CFLT have received respectively 700±225, 915±60 and 820±150 mg per day, resulting in mean GCV trough level of 0.75±0.5 mg/L. Lower doses registered in HT were adapted to RF. The safety data indicated that 9 neutropenias were recorded but only 2 were attributable to vGCV. Three D+/R– CFLT patients presented a positive pp65 Ag, 1 during the vGCV prophylaxis and 2 within the 4 months after discontinuation. 2 patients developed CMV disease, corresponding to an incidence of 6%. The TDM was performed to detect CMV reactivation to guide the treatment.

**Conclusion:** 300 mg GCV daily, adapted to RF appeared effective and safe for long CMV prophylaxis, related to efficient exposure to GCV in thoracic transplant patients. These first results also confirmed that the regular TDM is not necessary in case of oral vGCV prophylaxis for patients with stable RF.
**P1780** Clinical features and outcome of tuberculosis in solid-organ transplant recipients

N. Fernández-Sabé*, L. Lladó, S. Gil-Vernet, F. Alcaide, M. Santín, J. Carratalà (Barcelona, ES)

**Objectives:** The aim of this study was to analyze the frequency, characteristics, treatment and outcome of tuberculosis in solid-organ transplant (SOT) recipients.

**Methods:** We retrospectively reviewed medical charts of all cases of tuberculosis occurring in SOT recipients from 2000 to 2007. Data regarding baseline and clinical features, treatment and outcome were retrieved.

**Results:** A total of 13 of 1304 SOT recipients developed tuberculosis (1%). The frequency of tuberculosis according to the type of allograft was 1.3% (9 of 700) for kidney recipients, 0.8% (4 of 485) for liver recipients, and 0% (0 of 119) for heart recipients. Eight patients were males (61%) and the mean age was 55 years (range, 35–74 years). Seven patients were receiving more than one immunosuppressive drug by the time of diagnosis: corticosteroids (6), cyclosporine (5), mycophenolate mofetil (7), sirolimus (2) and tacrolimus (4). The mean time to the development of tuberculosis was 1726 days (range, 57–4131 days). Four patients (31%) developed tuberculosis within the first year post-transplantation. The mean duration of symptoms until diagnosis was 30 days (range, 1–180 days). Seven patients (54%) had pulmonary tuberculosis, 4 (31%) had disseminated infection and 2 patients (15%) had lymph nodes involvement. One patient had concomitant cytomegalovirus infection. No Mycobacterium tuberculosis strain was resistant to first-line antituberculous drugs. All patients were given isoniazid, and most of them received a 3-drug regimen. Rifampin was used in 9 cases. Six patients (4 liver and 2 renal recipients) developed hepatotoxicity, leading to discontinuation of antituberculous treatment in 4 cases. One patient developed rejection during treatment without allograft lost. Overall mortality was 15% (2 of 13 patients).

**Conclusions:** In this study, 1% of SOT recipients developed tuberculosis, which frequently presented with extra-pulmonary involvement and caused considerable mortality. Hepatotoxicity was a significant therapeutic drawback, mainly among liver transplant recipients.

**P1781** Need for a screening with antitoxoplasma IgG and IgM in transplantation

F. Genco, A. Di Matteo, E. Sarchi, L. Minoli, V. Meroni* (Pavia, IT)

**Objectives:** Toxoplasmosis is an opportunistic parasitosis that could be life-threatening in transplanted patients. The highest risk of infection and disease occurs in recipients with primary infection transmitted by a seropositive donor to a seronegative recipient (mismatch), but also as a reactivation of a previous infection. Screening for toxoplasmosis is mandatory and in mismatch recipients therapy is given immediately after transplantation. We have previously described the effectiveness of chemoprophylaxis and hygienic measures in in a cohort of donors and recipients of solid organ transplant referred to our Infectious Diseases Department. On the other hand, we noticed that presence of antitoxoplasma IgM in donors correlates with a higher seroconversion rate. Therefore, we suggest to screen donors not only for specific antitoxoplasma IgG but also for IgM.

**Patients and Methods:** We analyzed 1084 recipients and 540 donors with the following serological tests: CLIA IgG IgM, Toxo IgA (Dia Sorin Saluggia Italia), IgG ELFA (Biomerieux Marcy L’Etoile) Toxo IgM ISAGA, Toxo IgG Avidity (Biomerieux Marcy L’Etoile France), IgG IgM Western-Blot (LDBIO Lyon France). In all symptomatic cases nested PCR (Clinit Milano Italia) was performed on peripheral blood and cardiac biopsies.

**Results:** In our group of recipients seroprevalence was 56% and in the donors group it was 53%. Among these patients 1.7% (9) were IgM positive though IgG avidity test was low only in one case. The recipients of hearts from IgM positive donors were seropositive in 5 cases and seronegative in 4. Among these patients we recorded 2 seroconversions and 1 reactivation with ending of the patient.

**Conclusions:** The overall percentage of seroconversions was 11.8%, in mismatches it was 17.24%, but in transplanted patients with IgM positive donors seroconversion reached 50%. Furthermore, we observed 1 case of lethal reactivation. These preliminary data point out the different pattern of Toxoplasma infection (more frequent, more severe) when donors are IgM positive and therefore the need for testing for IgG and IgM. These findings also call for a more accurate follow-up in recipients of organs from an IgM positive donor even in presence of high avidity index.

**Clinical trials of antibiotics**

**P1782** Safety and efficacy of intravenous tigecycline in patients with bacteremia: pooled analysis from 8 phase 3 clinical trials

D. Gardiner*, G. Dukart, T. Babincha, A. Cooper (Collegeville, US)

**Background:** Tigecycline (TGC), the first approved glycylcycline antibiotic, has a broad spectrum of in vitro activity against both susceptible and multidrug-resistant bacteria. TGC has been studied for the treatment of complicated skin/skin structure infection (cSSSI), complicated intra-abdominal infection (cIAI) and community-acquired pneumonia (CAP), but data supporting its efficacy in patients with concomitant bacteraemia is limited.

**Methods:** Pooled data from patients with bacteraemia from 7 double-blind and 1 open-label trial of TGC compared with vancomycin/aztreonam, imipenem/ cilastatin, levofloxacin, vancomycin, or linezolid as standard therapies for cSSSI, cIAI, CAP, or serious infections due to vancomycin-resistant enterococcus, methicillin-resistant Staphylococcus aureus (MRSA), or resistant Gram-negative organisms were analyzed. The primary efficacy endpoint was the clinical cure rate at the test-of-cure assessment.

**Results:** A total of 190 patients with bacteraemia were identified (TGC n = 107; comparator n = 83). Mean Acute Physiology and Chronic Health Evaluation (APACHE) II scores (8.48 vs 7.38; p = 0.05) and body mass index (27.2 vs. 25.5; p < 0.045) were statistically significantly greater in the TGC than the comparator group; the groups were otherwise balanced with respect to demographic and clinical characteristics. Clinical cure rates were 76.6% and 77.1% for TGC and comparator, respectively (p = 1.000). Analyses by sex, age, creatinine clearance, primary infection site (cSSSI, cIAI, or CAP), APACHE score, and Fine score demonstrated clinical cure rates of 69% to 86% with no significant differences between TGC and comparator. Cure rates for diabetic patients were 11/21 (52.4%) and 12/15 (80.0%) for TGC and comparator, respectively (p = 0.1590). Clinical cure rates for the most commonly represented pathogens, S. aureus, Streptococcus pneumoniae, and Gram-negative species, were also not significantly different between treatment groups. No decrease in rate of cure was found in organisms with increasing TGC minimal inhibitory concentrations (MICs). The overall incidence of treatment-emergent adverse events was similar between TGC and comparator, with more gastrointestinal adverse events with TGC.

**Conclusions:** Tigecycline was effective and generally well tolerated in the treatment of bacteraemia associated with cSSSI, cIAI, and CAP, including MRSA infection. Cure rates were similar to those of comparative standard therapies.

**P1783** Comparison of the effects of ciprofloxacin, cotrimoxazole, amoxicillin, and chloramphenicol in patients with typhoid fever

I. Mahmoud*, K. Abdullah (Mosul, IQ)

**Objectives:** To compare the effects of ciprofloxacin, chloramphenicol, cotrimoxazole and amoxicillin in patients with typhoid fever.

**Methods:** Ninety three patients with symptoms and signs of typhoid fever and positive Widal test participated initially in the study. Seven patients were excluded from the study because of loss of follow-up and the final results were thus obtained from the remaining 86 patients.
Clinical trials of antibiotics

The main symptoms and signs reported at the start of the study were fever, headache, malaise, anorexia, abdominal pain, dry cough and splenomegaly. Widal test was positive in all patients which revealed 4 to 8 folds increase in the titer of antibodies against O and H antigens of Salmonella typhi.

The response to therapy was evaluated by: time taken to defervesce and the improvement of the patient’s condition. The later was defined as the improvement of the GIT symptoms, improvement or disappearance of headache, improvement of general condition of the patients, and the patient’s survival without a major complications.

Results: Duration of illness before antimicrobial administration were 10.43, 10.48, 11.15, and 10.64 days for ciprofloxacin, cotrimoxazole, amoxicillin and chloramphenicol respectively. No statistical differences in the duration of the illness were found before therapy among the 4 treatment groups.

The time taken for defervescence for ciprofloxacin group was 3.74 days, for amoxicillin group 5.9 days, for cotrimoxazole group 6 days and 5.2 days for chloramphenicol group. Time taken for defervescence was significantly shorter in those treated with ciprofloxacin (6.2 days) as compared with those taken cotrimoxazole, amoxicillin or chloramphenicol. Time taken for clinical improvement was significantly shorter in those patients given ciprofloxacin, amoxicillin or chloramphenicol. Time taken for clinical improvement was significantly shorter in those treated with ciprofloxacin (6.2 days) as compared with those taken cotrimoxazole (10.56days), amoxicillin (9.8 days) or chloramphenicol (8.32 days).

Conclusion: ciprofloxacin is a better drug for the treatment of patients with typhoid fever as it significantly reduces fever and other symptoms within a shorter time as compared with cotrimoxazole, amoxicillin or chloramphenicol.

P1784 Randomised clinical trial of short-course norfloxacin vs single dose fosfomycin for uncomplicated UTI in region with 10% resistance level of uropathogenic E. coli to fluoroquinolone

V Rafalskiy, L. Khodnevitch, I. Malev, A. Derevickiy (Smolensk, Tula, Roslavl, RU)

Objectives: Cut-off level of uropathogens resistance in the region is currently used in clinical guidelines (IDSA, 1999; EUA, 2006) as a selection criterion for some antimicrobials (co-trimoxazole – CTZ, fluoroquinolones – FQ). Our previous epidemiological study (2008) shows that in Smolensk region resistance of uropathogenic E. coli (UPEC) in community exceed 10% level. The aim of this study was to estimate of clinical outcomes after therapy by short course of FQ in patients with uncomplicated UTI in the region with community UPEC FQ-resistance >10%. As a comparator was selected antimicrobial with level of UPEC resistance equal 0% – fosfomycin tromitant.

Methods: A prospective controlled randomised study, including 108 women with uncomplicated UTI was carried out in primary health care centre. Women 18–55 years with signs and symptoms of lower UTI and signed informed consent were included in the study. We do not include women with upper, complicated or nosocomial UTI; duration of UTI >7 days; >2 relapses of UTI during last 6 months; antimicrobials treatment during last 30 days; hypersensitivity to FQ or fosfomycin; invasive urologic manipulation during last 30 days. Patients of the Group 1 were treated by norfloxacin 400mg twice a day for a 3 days, patients of the Group 2 were treated by one dose of fosfomycin tromitanate 3.0 g. The results of urine cultures and clinical investigations were collected on the days 5–7 (V2), 9–11 (V3) and 26–29 (V4) after first dose of medication.

Results: There were no differences between recovered uropathogens, age, and signs duration for patients of Group 1 and 2 (Table 1). Clinical improvement, cure and failure rate at V2 were 98.2%, 68.5%, 1.9% for Group 1 and 76%, 98%, 2.0% for Group 2. Eradication and persistence rate at V2 were 100% and 0% for Group 1, 95.8% and 4.3% for Group 2 (Table 1). There were no significant differences between efficacy and safety outcomes in Group 1 and 2.

Conclusions: Short course of FQ are effective for treatment of uncomplicated UTI even in region with UPEC resistance level exceed 10%. The results of antimicrobial susceptibility testing were not always related to the clinical outcome and bacterial resistance may overestimate the risk of therapeutic failure in UTI. Probably it is necessary more exactly to estimate and to use a resistance cut-off level for selection of antimicrobial for UTI.

Table 1. Characteristic of patients before treatment and key parameters of efficacy and safety

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients included</td>
<td>55</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Age, M±s</td>
<td>55.0±11.0</td>
<td>54.4±8.4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Duration of UTI, days, M±s</td>
<td>2.1±0.8</td>
<td>2.0±0.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Isolated recovered before treatment, n (%( )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>45/55 (85.5%)</td>
<td>30/49 (79.5%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>3/55 (5.5%)</td>
<td>3/49 (6.1%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>2/55 (3.5%)</td>
<td>2/49 (4.1%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>1/55 (1.9%)</td>
<td>1/49 (2.0%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Other</td>
<td>2/55 (3.5%)</td>
<td>3/49 (6.1%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Microbiological efficacy, n (%( )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli eradication rate (on V2)</td>
<td>54/55 (98%)</td>
<td>40/49 (81.6%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Persistence (on V2)</td>
<td>9/55 (16.4%)</td>
<td>13/49 (26.5%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Remission (on V3 or V4)</td>
<td>1/2 (50%)</td>
<td>1/2 (50%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Relapse (on V3 or V4)</td>
<td>1/2 (50%)</td>
<td>1/2 (50%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Clinical efficacy, n (%( )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cure (on V2)</td>
<td>37/55 (67.3%)</td>
<td>36/49 (73.5%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Improvement (on V2)</td>
<td>53/55 (96.4%)</td>
<td>49/49 (100%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Failure (on V2)</td>
<td>2/55 (3.8%)</td>
<td>2/49 (4.1%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Relapse (on V3 or V4)</td>
<td>3/35 (8.6%)</td>
<td>1/20 (5%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Adverse events, n (%( )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

P1785 Efficacy of IV/oral moxifloxacin in the treatment of complicated skin and skin-structure infections: results of the RELIEF study


Objectives: Selection of the optimal antimicrobial for treatment of complicated skin and skin structure infections (cSSSIs) can be difficult. A number of IV antimicrobials have been investigated for the management of cSSSIs. Amongst them, moxifloxacin (MXF) has been shown to have efficacy similar to standard therapies. The RELIEF study was conducted to provide additional data in well-characterised patients with confirmed cSSSIs.

Methods: RELIEF was a prospective, randomised, double-dummy, double-blind, multinational, multicentre study enrolling patients with a diagnosis of major abscess, diabetic foot infection, wound infection or infected ischaemic ulcer. Patients were stratified according to infection severity, requirement for surgery, and cSSSI diagnosis, and randomised to IV/PO MXF 400 mg qd or piperacillin/tazobactam 4.0/0.5g tds followed by PO amoxicillin-clavulanic acid (PIP/TAZ-AMC) 875/125 mg bd, for 7–21 days. The primary efficacy variable was clinical response 14–28 days after completion of therapy. Non-inferiority of MXF was demonstrated if the lower limit of the 95% confidence interval (CI) was above −10%.

Table: Clinical and bacteriological response in the different patient populations of the RELIEF study

<table>
<thead>
<tr>
<th>Populations</th>
<th>MXF n (%( )</th>
<th>PIP/TAZ-AMC n (%( )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per-protocol</td>
<td>322/363 (88.7%)</td>
<td>275/307 (89.6%)</td>
</tr>
<tr>
<td>MBV</td>
<td>235/270 (87.0%)</td>
<td>218/243 (85.8%)</td>
</tr>
<tr>
<td>ITT</td>
<td>353/426 (82.9%)</td>
<td>305/377 (80.9%)</td>
</tr>
<tr>
<td>ITT with organisms</td>
<td>256/313 (81.8%)</td>
<td>234/290 (80.7%)</td>
</tr>
<tr>
<td>Bacteriological response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBV</td>
<td>224/270 (83.0%)</td>
<td>210/243 (86.4%)</td>
</tr>
<tr>
<td>ITT with organisms</td>
<td>244/313 (78.0%)</td>
<td>227/290 (78.3%)</td>
</tr>
</tbody>
</table>

ITT = invasive skin and soft tissues infection; MBV = moderately severe skin and soft tissue infection; PIP/TAZ-AMC = piperacillin/tazobactam; RX = moxifloxacin.
**Results:** Of 813 patients randomised (MXF=432, PIP/TAZ-AMC=381), 803 were valid for ITT/safety analyses (MXF=426, PIP/TAZ-AMC=377). In the PP population (MXF=363, PIP/TAZ-AMC=307), diagnoses were: abscess (n=320, 47.8%), diabetic foot infection (n=207, 30.9%), wound infection (n=110, 16.4%), and infected ulcer (n=33, 4.9%). The most frequent individual pathogens in the microbiologically valid (MBV) population were: methicillin-susceptible Staphylococcus aureus (n=308), Escherichia coli (n=113), Enterococcus faecalis (n=110), Streptococcus pyogenes (n=60) and Bacteroides fragilis (n=44). For the primary efficacy variable (clinical response at TOC), MXF was non-inferior to PIP/TAZ-AMC (Table). Good bacteriologic efficacy was also seen (Table). In the ITT/safety population, incidences of treatment-emergent adverse events, and treatment-emergent drug-related adverse events were similar in the MXF and PIP/TAZ-AMC groups (23% vs 19%, P=0.14, and 9% vs 7%, respectively). In this large multicentre study, IV/PO MXF was clinically non-inferior to IV PIP/TAZ-AMC in the treatment of patients with cSSSIs. Both treatments were well tolerated. Safety profiles of study regimens were similar. These data confirm the efficacy of IV/PO MXF for the treatment of cSSSIs.

### Table: Clinical and bacteriologic responses at TOC

<table>
<thead>
<tr>
<th></th>
<th>MXF</th>
<th>PIP/TAZ</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per-protocol</td>
<td>160/167 (95.8)</td>
<td>147/153 (96.1)</td>
<td>−4.2, 4.5</td>
</tr>
<tr>
<td>MBV</td>
<td>119/125 (95.2)</td>
<td>113/117 (96.6)</td>
<td>−5.5, 4.3</td>
</tr>
<tr>
<td>ITT</td>
<td>163/183 (89.1)</td>
<td>151/169 (89.3)</td>
<td>−5.6, 7.2</td>
</tr>
<tr>
<td>ITT with organisms</td>
<td>122/135 (90.4)</td>
<td>114/125 (91.2)</td>
<td>−5.6, 8.1</td>
</tr>
<tr>
<td>Bacteriological response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBV</td>
<td>117/125 (93.6)</td>
<td>113/117 (96.6)</td>
<td>−7.5, 3.1</td>
</tr>
<tr>
<td>ITT with organisms</td>
<td>119/135 (88.1)</td>
<td>114/125 (91.2)</td>
<td>−8.0, 6.3</td>
</tr>
</tbody>
</table>

**Conclusion:** Efficacy of IV/oral moxifloxacin and IV piperacillin/tazobactam followed by oral amoxicillin-clavulanic acid in the treatment of major abscesses: results of the RELIEF study

I.C. Gyssens*, M. Dryden, P. Kujath, D. Nathwani, N. Schaper, P. Arvis, P. Reinmuth, J. Alder, B. Hampel (Nijmegen, Maastricht, NL; Winchester, Dundee, UK; Lubeck, Berlin, Wuppertal, DE; Lille, FR; Pine Brook, US)

**Objectives:** Major abscesses often need significant surgical intervention and antimicrobial therapy. Depending on their location, bacterial aetiology is variable or polymicrobial. Fluoroquinolones – such as moxifloxacin (MXF) – may offer advantages over other antimicrobial classes due to their broad spectrum and pharmacodynamic properties. MXF is approved in some European countries for the treatment of complicated skin and skin structure infections (cSSSIs) but due to limited data the approval does not include major abscesses. The RELIEF study was conducted to provide further data on the efficacy of MXF in specific cSSI diagnoses. Data on major abscesses are presented.

**Methods:** This was a double-dummy, double-blind, randomised, controlled trial. Patients with a major abscess associated with extensive cellulitis and requiring antimicrobial therapy in addition to surgical drainage. Mean (SD) lesion area was (MXF) 111 (141) cm² and of the buttocks (36.1%) were most frequent. MXF was non-inferior to PIP/TAZ-AMC with respect to clinical response at TOC (Table). Bacteriologic success rates were also comparable.

**Conclusion:** IV/PO MXF was non-inferior to IV PIP/TAZ-AMC in the patients with major abscesses. Based on these results, MXF can be considered a valuable option for the treatment of major abscesses.
**P1788** Catheter exit site infections in patients undergoing continuous ambulatory peritoneal dialysis


**Objectives:** (a) To study the bacterial and fungal causes of catheter exit site infections in CAPD patients, the frequency of isolations and the susceptibility patterns to antimicrobial agents. (b) To evaluate the frequency of catheter exit-site infection-related peritonitis and (c) to determine the refractory cases to treatment and the frequency of catheter loss.

**Material-Methods:** We reviewed all the cases of catheter exit-site infections from January 2002 through December 2007. Smear or purulent exit-site drainage of patients with symptoms and signs of exit site inflammation were cultured for aerobic and anaerobic bacteria and fungi with the standard laboratory methods. Antibiotic susceptibility testing was performed by the disk diffusion technique, the Vitek-2 system and the Etest.

**Results:** Ninety one episodes of culture-positive exit-site infection occurred in 41 CAPD patients. Pseudomonas aeruginosa, Gram-negative (A. baumannii, A. Iwoffii, P. mirabilis, E. cloacae, S. marcescens, K. pneumoniae, E. coli, Morganella morganii, Moraxella, P. putida, Sphingomonas paucimobilis, Acrobacter xylosoxidans), mixed infections caused by Gram-negative and Gram-positive bacteria (S. epidermidis, Bacillus spp, Corynebacterium spp), coagulase-negative staphylococci (CoNS), Staphylococcus aureus and Candida spp were identified in 9 (9.9%), 18 (19.78%), 7 (7.7%), 34 (37.36%), 6 (6.6%), and 2 (2.19%) cases respectively. The susceptibility testing showed a high resistance rate of S. epidermidis to methicillin (60.5%) and a low resistance rate of S. aureus (16.7%). A Corynebacterium group 1 was susceptible only to glycopeptides and rifampicin. All Gram-negative bacteria were multiresistant except for A. baumannii and S. maltophilia.

**Conclusions:** (a) CoNS are the most common cause of exit-site infection followed by P. aeruginosa and S. aureus. (b) P. aeruginosa and Candida exit-site infections are refractory to treatment and may lead to catheter loss. (c) Peritonitis associated with exit-site infections occurs in low rate and is caused by Gram-negative bacteria and CoNS. (d) In case of empirical treatment the high resistance rate of CoNS to methicillin and the high frequency of P. aeruginosa as a cause of exit site infection should be taken in to account. (e) The empirical therapy for severe catheter exit site infections in CAPD patients should be adapted to the local epidemiology (frequency of causative agents and their susceptibility pattern).

**P1790** Effect of intermittent moxifloxacin therapy on the microbiology of sputum cultures from patients with chronic obstructive pulmonary disease (the PULSE study)

S. Sethi*, J. Alder for the PULSE Study Group

**Objectives:** The PULSE study has demonstrated the efficacy and safety of intermittent pulsed therapy of moxifloxacin (MXF) (400 mg PO q.d. for 5 days every 8 weeks for 6 cycles) in the reduction in the number of exacerbations of chronic obstructive pulmonary disease (COPD). Intermittent therapy was used to reduce the potential for emergence of resistant isolates associated with chronic, daily therapy. We now present the results of sputum microbiological analysis [microbial identification, eradication, and changes in minimal inhibitory concentration (MIC)] carried out during the 72-week study in patients treated with MXF or placebo.

**Methods:** Sputum samples were collected from all 1149 patients in the ITT population at all clinic visits. MXF susceptibility testing was performed for Haemophilus spp., Streptococcus pneumoniae, Moraxella catarrhalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus from both treatment arms. MIC to a range of antibacterial agents was determined by broth microdilution. Frequency of isolation and changes in MIC distributions of specific pathogens were compared for the MXF and placebo arms, among patients colonised with these pathogens at randomisation.

**Results:** The most frequent isolates at randomisation were H. influenzae, H. parainfluenzae, S. pneumoniae, S. aureus and P. aeruginosa. MXF therapy achieved eradication in a majority of the patients colonised by H. influenzae, H. parainfluenzae, and S. pneumoniae, but not in those colonised by S. aureus or P. aeruginosa. Spontaneous eradication was seen at a significantly lower frequency in the placebo group (Table).
success rate was 91% (35% cured, 55% improved). The success rates by infection type were: BAC (46/50; 92%), SSTI (46/51; 90%), and IE (9/13; 69%); success was not different by dose (≥6 to <8 vs. ≥8 mg/kg).

**Conclusion:** Daptomycin was well tolerated and effective in BAC, SSTI, and IE at doses of ≥6 mg/kg. Further studies of daptomycin at doses above 6 mg/kg are warranted.

**Results:** Of 694 enrolled patients, 348 received CPT and 346 V/A. Baseline characteristics of treatment groups were comparable. Clinical cure rates were similar for CPT and V/A in CE (92.2%, 271/294 vs. 92.1%, 269/292; 95% CI, 0.94 to 1.05) and MITT (85.1%, 291/342 vs. 85.5%, 289/338; 95% CI, 0.58 to 0.70) populations, respectively. Clinical cure rate for MRSA cSSSI was 91.4% (64/70) for CPT and 93.3% (56/60) for V/A. Microbiological success rate was also similar for CPT and V/A overall and for MRSA. The rates of AEs, SAEs, deaths and discontinuations due to AEs were similar for CPT and V/A. Most common AEs for CPT and V/A were diarrhea (6.5% vs. 4.4%), nausea (6.2% vs. 5.6%), headache (5.3% vs. 5.3%), and pruritus (3.8% vs. 8.3%), respectively.

**Conclusions:** CPT had high clinical cure and microbiological success rates, was efficacious against MRSA and other common cSSSI pathogens, and was well tolerated. CPT has the potential to provide a monotherapy alternative for treatment of cSSSI.

**Objective:** Antibiotic safety is a major determinant in selecting osteomyelitis therapy. Recent publications highlight the preliminary experience with daptomycin in the treatment of osteomyelitis and orthopedic-related infections. Additional data describing the long-term safety of daptomycin treatment is valuable.

**Methods:** Data were collected as part of the Cubicin Outcomes Registry and Experience (CORE) program, a retrospective, observational, multicentre study, to describe the clinical use of daptomycin. Efficacy at the end of daptomycin therapy was determined by each centre's investigator as cured, improved, failure, or nonevaluable. Patients (pts) who had a diagnosis of osteomyelitis were selected from the combined 2005 and 2006 CORE database as the safety population. Pts who had an evaluable clinical outcome, received greater than 3 days of daptomycin therapy, and had appropriate final dose adjustment for renal function were included in efficacy population.

**Results:** Three hundred twenty-seven pts met study criteria for safety, 188 (57%) received ≥6 mg/kg and 139 (43%) received <6 mg/kg. Two hundred and twenty-two (68%) pts received daptomycin for 21 days or more. Thirty-one (10%) pts experienced adverse events classified as possibly related to daptomycin. Serious adverse events were reported less frequently in pts receiving ≥6 mg/kg (4%, 8/188) than those receiving <6 mg/kg (9%, 13/139); P = 0.07. The incidence of adverse events classified as possibly-related, whether serious or non-serious, were similar regardless of daptomycin dose. No difference was observed in the rate of CPK elevations by daptomycin dose 9/188 (5%) ≥6 mg/kg; 6/139, (4%) <6 mg/kg. Pts receiving final doses of 6 mg/kg or more showed a trend of higher improved rates (96%, 137/143) than those receiving lower doses (90%, 96/107), P = 0.08.

**Conclusion:** These data suggest that daptomycin is well-tolerated at higher dosages and for the longer therapy durations needed for osteomyelitis. Doses of daptomycin of 6 mg/kg or greater may be associated with greater clinical improvement. Ideally, these results should be confirmed via a prospective clinical trial.

**Objective:** Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a common cause of complicated skin and skin structure infections (cSSSI). Increasing antibiotic resistance and significant morbidity in cSSSI have led to a need for new effective and safe therapies. Ceftaroline (CPT), a novel parenteral cephalosporin with excellent in vitro activity against MRSA, multi-drug resistant *Streptococcus pneumoniae*, and many Gram-negative pathogens, was evaluated as therapy of cSSSI. The primary objective was to determine noninferiority (lower limit of 95% CI –10%) in clinical cure rate of CPT to vancomycin plus aztreonam (V/A) in clinically evaluable (CE) and modified intent-to-treat (MITT) populations.

**Methods:** Adults patients with cSSSI requiring IV therapy received CPT 600 mg q12h or V 1 g plus A 1 g q12h for 5–14 days (randomised 1:1). Clinical and microbiological response, adverse events (AEs), and laboratory tests were assessed.

**Results:** Of 694 enrolled patients, 348 received CPT and 346 V/A. Baseline characteristics of treatment groups were comparable. Clinical cure rates were similar for CPT and V/A in CE (92.2%, 271/294 vs. 92.1%, 269/292; 95% CI, –0.4 to 0.45) and MITT (85.1%, 291/342 vs. 85.5%, 289/338; 95% CI, –5.8 to 5.0) populations, respectively. Clinical cure rate for MRSA cSSSI was 91.4% (64/70) for CPT and 93.3% (56/60) for V/A. Microbiological success rate was also similar for CPT and V/A overall and for MRSA. The rates of AEs, SAEs, deaths and discontinuations due to AEs were similar for CPT and V/A. Most common AEs for CPT and V/A were diarrhea (6.5% vs. 4.4%), nausea (6.2% vs. 5.6%), headache (5.3% vs. 5.3%), and pruritus (3.8% vs. 8.3%), respectively.

**Conclusions:** CPT had high clinical cure and microbiological success rates, was efficacious against MRSA and other common cSSSI pathogens, and was well tolerated. CPT has the potential to provide a monotherapy alternative for treatment of cSSSI.
Randomised, placebo-controlled phase III trial of docetaxel plus carboplatin with or without levofloxacin prophylaxis in elderly patients with advanced non-small cell lung cancer: The APRONTA Trial

N. Dickgreber*, S. Nagel, K. Roscher, W. Schuette (Hannover, Halle, Berlin, DE)

Objectives: Elderly patients receiving chemotherapy are more likely than younger patients to experience febrile neutropenia and infection. Prophylactic fluoroquinolone administration during chemotherapy reduces the rate of febrile neutropenia, infection and hospitalisation vs placebo. The aim of this study was to examine the effect of levofloxacin prophylaxis on infection rates during chemotherapy with docetaxel plus carboplatin in elderly patients with advanced non-small cell lung cancer (NSCLC).

Methods: This was a randomised, double-blind, placebo-controlled Phase III study. Patients aged ≥65 years with previously untreated, histologically/cytologically proven stage IIIB/IV NSCLC and normal cardiac, renal, hepatic and haematological function were included. Active infection or antibiotics 72 hours before inclusion was not permitted. Patients were randomised to receive docetaxel (75mg/m² intravenous [IV], Day 1) plus carboplatin (AUC 6 IV, Day 1) every 3 weeks, plus either placebo or levofloxacin (500 mg oral [po], once daily) on Days 5–11. The primary endpoint was grade 3/4 infection rate or grade 1/2 infection rate with infection therapy.

Results: Overall, 192 patients (median age 70 years; 80% male; Eastern Cooperative Oncology Group performance status 0/1/2 in 36%/55%/9%) were randomised to docetaxel plus carboplatin and either levofloxacin (n = 99) or placebo (n = 93); 5 patients received no treatment and were excluded from the ITT population. The rate of grade 3/4 infection was 27.5% (95% CI: 19.3–39.0%) with levofloxacin vs 36.7% (95% CI: 27.1–48.0%) with placebo. Median time to first infection was 67 days for levofloxacin vs 46 days for placebo. The between-group difference in median time to first infection was greater for patients aged over 70 years (69 vs 27 days for levofloxacin and placebo, respectively). Grade 3/4 infection occurred in 8% of patients receiving levofloxacin vs 26% of patients receiving placebo; there was one grade 5 infection in each group. Pneumonia and sepsis occurred in 12% and 1% of levofloxacin patients, respectively, vs 22% and 4% of the placebo group. Grade 3/4 mucositis, nausea and vomiting occurred in 5%, 3% and 3% of levofloxacin patients, respectively, vs 0%, 1% and 0% of placebo patients. Efficacy was similar in both groups.

Conclusions: Levofloxacin prophylaxis is well tolerated in elderly patients receiving docetaxel plus carboplatin chemotherapy and reduces the rate of infection compared with placebo.

Biofilms

Comparative antimicrobial susceptibility of biofilm versus planktonic forms of Salmonella spp. from children with gastroenteritis

K. Papavasileiou*, H. Papasavasileiou, A. Voyatz, S. Chatzipanagiotou (Athens, GR)

Biofilms are involved in a variety of bacterial and fungal infections. Biofilm bacteria produce an extracellular polymeric substance (EPS), which protects them against antimicrobial agents, thus leading to multi-drug clinical resistance and therapeutic failure. Numerous studies have shown that Salmonella is capable of adhering and forming biofilms on metal, glass, or rubber surfaces.

Objective: This study aimed to detect the production of biofilms by clinical strains of Salmonella spp. isolated from children with gastroenteritis and to compare the antimicrobial susceptibility of planktonic versus biofilm forms.

Methods: During a three year period (2005–2007) 194 strains of Salmonella were collected from hospitalised children with gastroenteritis as well as from children presenting at the outpatient department, aging from 1–14 years. The isolation and identification of Salmonella spp. was performed by conventional bacteriological methods. MIC was determined by the Broth Dilution Method (macrodilution) according to the guidelines of CLSI. Biofilm formation was detected by using silicone disks. The strains producing biofilms were further tested for their antimicrobial susceptibility by using a modified broth dilution method.

Results: Biofilm formation was detected in 109 out of 194 Salmonella strains (56%). Planktonic bacteria were significantly more susceptible to the antimicrobials as compared to the biofilm bacteria. The resistance rates respectively for the planktonic and the biofilm forms were as follows: gentamicin 0% and 89.9%, ampicillin 12.8% and 84.4%, coamoxiclav 0% and 51.4%, ciprofloxacin 0.9% and 63.3%, ceftaxime 7.4% and 63.3%, cefotaxime 0.9% and 23.8%, imipenem 0% and 7.3%, ciprofloxacin 0% and 2.8%, moxifloxacin 0% and 2.8%.

Conclusions: The great majority of the biofilm forms were susceptible to the fluoroquinolones, while they showed high level resistance to ampicillin, coamoxiclav and cefotaxime. The present study demonstrated a high rate of biofilm production among the clinical isolates of Salmonella spp., as well as a significant association of the biofilm forms with increased antimicrobial resistance. This phenomenon might be a cause of clinical therapeutic failure in Salmonella infections, despite the in vitro antimicrobial susceptibility of the causative bacterial strains.
Evaluation of aggregative adherence and biofilm formation in β-lactamase producing Proteus mirabilis isolates from different clinical settings in Italy


Objectives: P. mirabilis is the second most relevant uropathogen and urinary tract infections (UTIs) caused by TEM-92 ESBL and CMY-16 CBBL producers are increasing in Italian settings. In catheterised patients UTI may become chronic, with a microbial persistence maybe due to both antibiotic resistance and adherence. We investigated the biofilm formation and the aggregative adherence of TEM-92 and CMY-16 producers.

Methods: 20 epidemiologically distinct clinical isolates of P. mirabilis collected during 2003–2007 from Italian hospitals and nursing homes, including CMY-16 and TEM-92 producers and β-lactamase (BL) negative strains, were examined for biofilm formation by crystal violet assay in different culture conditions and in presence of imipenem (IMP) or piperacillin/tazobactam (TZP) sub-MIC concentrations. All strains were susceptible to IMP and TZP by conventional tests. The presence of mrrA gene encoding for the major fimbrial subunit of MR/P fimbriae was investigated by PCR. 4 selected strains were also tested for adherence to LL-CMK2 epithelial cells grown on a coverslip.

Results: All strains resulted proficient in biofilm formation which was favoured by nutrient-deficient medium (urine). Biofilm formation was similar for CMY-16 and TEM-92 producers; on the contrary, a fewer biofilm formation was observed in BL negative strains. Sub-MIC concentrations of IMP and TZP stimulated biofilm increase in all strains. mrrA gene was detected in all strains; the 4 strains studied for adherence to LLC-MK2, after 1 h of incubation, showed few bacteria forming aggregates in intercellular spaces and 75% of bacteria adhered to the coverslips, instead after 4 hrs great adherence in intercellular spaces and few bacteria on cells, especially arranged in chains along the border of the cytoplasm, were observed. We found differences in arrangement on coverslips among the 4 strains tested.

Conclusions: All BL producers, regardless of the enzyme type, resulted equally proficient in biofilm production that increased in presence of sub-MIC concentrations of IMP and TZP [β-lactams to which they resulted susceptible; while the BL negative strains showed a low ability to produce adhesion factors. Cellular adherence assays showed a preferential adhesion trend to inert surfaces rather than to epithelial cells. Although the results didn’t fully support a direct correlation between BL production, biofilm and persistence, both these mechanisms contribute to UTIs chronicness.

Bioelectric effect decreases alginate production in Pseudomonas aeruginosa biofilms

K. Hamdi, A. Shoaie Hassan* (Tehran, Fars, IR)

Objectives: Low electrical currents are able to increase biocides activity against Gram-negative bacteria, especially in biofilms. Here we report the enhanced activity of effective antibiotics by bioelectric effect against the more resistant biofilm of Pseudomonas aeruginosa due to inhibiting the extracellular alginate polymer production.

Methods: Pseudomonas aeruginosa biofilm was designed on a membrane suspended between two electrode plates in an electrical colonisation cell. Amikacin (4 mg g⁻¹) and gentamicin (10 mg g⁻¹) were attempted on biofilms at ten times of their MIC for 24 h in the presence of 0 and 9 mA cm⁻² current density. The alginate production was measured at these concentrations and in combination with bioelectric effect. Cultures were stirred with a magnetic bar for 3 to 5 h, and bacterial cells were removed by centrifugation for 1 h at 18,000 × g at 4°C. The clear supernatant was heated for 30 min at 80°C to kill viable bacteria and passed through a 0.45 μm filter. Crude alginate was precipitated from the supernatant by addition of cold absolute ethanol to a final concentration of 80% (v/v). The results were compared in pairs using the SPSS method. For significant differences, P ≤ 0.05.

Results: The antibiotics alone reduced the biofilm population and in the presence of bioelectric effect the viable population was further reduced by gentamcin and especially by amikacin due to 47% and 60% reduction in alginate production, respectively.

Alginic production in P. aeruginosa strain 8821 after treatment with 0 and 9 mA/cm² electrical current in combination with antibiotics.

<table>
<thead>
<tr>
<th>Electric current</th>
<th>Antibiotics</th>
<th>Time</th>
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<th>16</th>
<th>24</th>
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</thead>
<tbody>
<tr>
<td>0 mA/mm²</td>
<td>Amikacin</td>
<td>1.44</td>
<td>1.11</td>
<td>0.63</td>
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<tr>
<td>DC</td>
<td>Gentamicin</td>
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<td>1.64</td>
<td>0.91</td>
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<td>1.92</td>
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<tr>
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</tr>
</tbody>
</table>

Conclusion: The bioelectric effect reduced the alginate production in P. aeruginosa and enhanced the penetration of effective antibiotics into the biofilms.
Mixed biofilms formed by *Haemophilus influenzae* and *Streptococcus pneumoniae* and expression of certain biofilm-related genes

E. Kalmuni, P. Asumaniemi, T. Kajalainen, T. Tapiainen, M. Uharti, M. Leinonen, A. Saukkoriipi* (Oulu, FI)

**Objectives:** Nontypeable *Haemophilus influenzae* (NTHi) and *Streptococcus pneumoniae* (Pnc) cause otitis media, which has been suggested to be caused by bacteria residing in biofilms. Both NTHi and Pnc have been shown to be able to form biofilms. We studied biofilm formation of mixed cultures of Hi and Pnc in vitro and the expression of certain genes that have been associated to the biofilm form of growth in these bacteria.

**Methods:** Four *H. influenzae* (Hi; three NTHi and one type b) and 4 Pnc isolates (two unencapsulated, two encapsulated) were grown separately and combined on polystyrene microwell plates for 5, 18, and 22 h. For each time point there was one plate for crystal violet staining and another for RNA extraction. All strains and combinations were tested in triplicate. The expression levels of peroxiredoxin-glutaredoxin (pdgX) and outer membrane protein P6 genes of Hi and autolysin (lytA) and penicillin-binding protein 2x (pbp2x) genes of Pnc were studied by relative quantitative PCR where the 16s rRNA genes of Hi and Pnc were used as reference genes.

**Results:** At 18 h, the unencapsulated Pnc isolates formed biofilms detectable by crystal violet staining whereas the Hi isolates did not. When an unencapsulated Pnc was grown together with an NTHi and Hib isolate, the OD540 value of the biofilm decreased from 0.31 to 0.07 and 0.09. However, when the unencapsulated Pnc was grown together with a capsulated Pnc, no difference was seen. Differences in gene expression were seen at 5 h. The mean expression levels of P6 were 3.79 and 3.32 in two of the nontypeable Hi isolates and 0.20 in the Hib isolate. When an unencapsulated Pnc isolate was grown with the NTHi and Hib, the mean expression level of P6 was 3.98 and 3.26, respectively. When the NTHi and Hib isolates were grown together, no increase in the expression levels were seen as compared to the NTHi alone (3.94 vs. 3.79). The expression of lytA and pbp2x at 5 h in one of the unencapsulated Pnc isolates were 1.38 and 0.59, respectively. When an NTHi or Hib was grown together with this isolate, the expression of lytA decreased to 0.41 and 0.37, respectively, and pbp2x to 0.13 and 0.26, respectively.

Expression of these two genes in the other Pnc isolates was below 0.5 when grown separately and together with Hi.

**Conclusion:** Unencapsulated Pnc and Hi affect each other’s biofilm formation and gene expression in vitro. Mixed biofilms may play a role e.g. in the pathogenesis of otitis media.

In vitro interference of cefditoren on slime production by *Streptococcus pneumoniae*


**Objectives:** To explore the in vitro effect of cefditoren (CDN) on slime production by *Streptococcus pneumoniae* strains belonging to different serotypes and with different CDN MICs.

**Methods:** Eleven *S. pneumoniae* isolates with the following CDN MICs (mg/l) were used: 0.015 (serotypes 23A and 21), 0.03 (serotype 31), 0.06 (serotype 42), 0.25 (serotypes 6B and 35B) and 0.5 (serotypes 9V – 2 strains – 14, 19A and 23F). Slime production was assessed as previously described (J Clin Microbiol 1985;22:996–1006), with modifications, in microtiter plates with HTM in the absence of CDN (basal) and in the presence of CDN 0.03 mg/l, by measuring optical density (OD) at 450 nm using an spectrophotometer. *S. pneumoniae* R6 was used as positive control and non-incubated HTM broth as negative control. All experiments were performed 12 times and mean values were considered.

**Results:** The Figure shows mean OD corresponding to basal determinations (triangles, continuous line) and determinations in the presence of CDN (squares, dotted line) for the eleven strains distributed by CDN MIC.

**Conclusion:** CDN at supra-inhibitory concentration (strains with MIC of 0.015 mg/l), inhibitory concentration (strain with MIC of 0.03 mg/l) or sub-inhibitory concentration (strains with MIC of 0.06–0.5 mg/l), reduced slime production in slime-producing *S. pneumoniae* strains, regardless the serotype or the magnitude of basal slime production.

Macrophase interactions with biofilm-producing *Staphylococcus epidermidis*

A. Spiliopoulou, F. Kolontisou, M. Krevvata, I. Spiliopoulou, G. Dimitracopoulos, E.D. Anastassios* (Patras, GR)

To compare immune responses of biofilm-producing *S. epidermidis* existing either in planktonic or in biofilm phase in terms of IL-12 production, bacterial adhesion on macrophages and resistance to phagocytosis process. ATCC35983 and two clinical biofilm-positive, ica-positive *S. epidermidis* strains were used. Planktonic phase bacteria were obtained after 2h incubation in Tryptic Soy Broth (TSB); whereas biofilm phase bacteria after 24h incubation of bacterial suspensions and homogenisation of bacterial cells embedded in biofilm attached to the bottom of the tube. Monocytes were separated from human peripheral blood mononuclear cells by plastic adherence and differentiated in macrophages. Phagocytosis experiments were performed by co-incubation of cells with bacteria at 1:10 ratio for 20, 60, 90 and 120 min; removal of extracellular bacteria and further incubation in antibiotic supplemented medium. Intracellular bacteria were counted by serial dilutions on blood agar plates. For measuring bacterial adhesion to macrophages, a modified ELISA was used where macrophages cultivated on 96-well plates were incubated with biotinylated bacterial suspensions. For cytokine determination, macrophages cultivated on 24-well plates were stimulated with bacteria at 1:10 and 1:25 ratio for 45 min, extracellular bacteria were removed and macrophages were further incubated for 12 h. IL-12p40 concentrations were measured in supernatants by commercial ELISA kit.

Biofilm phase bacteria showed increased adhesion on macrophages compared to planktonic phase bacteria (7.6±0.02×10^6 for biofilm phase bacteria vs 3±0.06×10^6 CFU/macrophase monolayer for planktonic phase bacteria). Biofilm phase bacteria were internalised in greater proportion (10-fold) than planktonic phase bacteria and showed higher degree of intracellular survival (Table 1). Planktonic phase bacteria elicited higher amounts of IL-12p40 than biofilm phase bacteria (planktonic phase 645±95 and 1,029±48 pg/ml vs biofilm phase 231±72 and 396±26 pg/ml for 1:10 and 1:25 ratio respectively) (p < 0.05).

Biofilm phase bacteria are efficiently internalised but seem to be more resistant to killing by macrophages than their planktonic counterparts. Internalisation of biofilm phase bacteria does not promote IL-12 production and macrophages can not manage an efficient Th1 response.
These findings could contribute to insight mechanisms of resistance of biofilm-associated infections to immune system responses.

**P1803** The effect of different proteases on staphylococcal biofilm


**Objectives:** The metalloprotease serratiopeptidase (SPEP) has been proved to be effective in the treatment of biofilm of different Gram-positive bacterial species belonging to the *Listeria* and *Staphylococcus* genera. Furthermore, SPEP has been used as an anti-inflammatory agent for over 30 years. The analysis of surface protein profiles of treated and untreated bacteria revealed that SPEP modulates specifically the protein pattern acting on different adhesins and autolysins. In order to highlight the mechanism of action of SPEP, different proteases, including metalloproteases and serin-proteases, were tested and compared for their capability to impair biofilm formation and to modulate protein expression of different staphylococcal strains.

**Methods:** Six *Staphylococcus* strains were studied, 3 *S. epidermidis* and 3 *S. aureus*. Biofilm formation and surface protein pattern was evaluated in the presence of the following proteases: SPEP, carboxypeptidase-A, proteinase-K, trypsin, chymotrypsin. Biofilm growth was assessed by the Christensen method. Proteins were analyzed by SDS-PAGE and zymography to evaluate modifications of the expression of autolytic enzymes. The presence of ica locus and of genes involved in adhesion and autolytic pattern was revealed by PCR. agr-typing was carried out by RT-PCR.

**Results:** The effect of tested proteases was not related to the protease category but was strain-dependent. SPEP, reducing the biofilm growth by RT-PCR.

**Conclusion:** The degradation of staphylococcal surface proteins does not necessarily result in biofilm inhibition. Moreover, the action of proteases is not related to the presence of icaADCB locus. In the strain more sensitive to SPEP action, *S. aureus* 653P, we investigated the agrD expression, which markedly increases following SPEP treatment.

**P1804** Sterilisation of staphylococcal biofilms with delta-toxin plus rifampin in a rat model

**V. Pintens, R. Merckx, M. Shahrooei**, J. Van Eldere (Leuven, BE)

**Objectives:** Biofilm-associated staphylococci are resistant to antibiotics, making eradication difficult. This may partly be due to low metabolic activity of sessile bacteria. The agr (accessory gene regulator) operon is a quorum-sensing system and may be involved in detachment of staphylococci from biofilm. We have previously shown that the agr-encoded delta-toxin increases agr expression and metabolic activity in biofilm-bacteria. Here we investigated the effect of delta-toxin alone and combined with rifampin on persistence of established *S. epidermidis* biofilms in vitro and in vivo.

**Methods:** In vitro biofilms were grown in 96-well plates. Biofilm formation was quantified optometrically with 1% crystal violet. For in vivo experiments we used a previously described rat model with subcutaneously implanted catheters; the amount of biofilm-bacterium was measured 24 hr after administration unless otherwise specified.

**Results:** In vivo, treatment of 1-day old biofilms with 10 μg/ml delta-toxin reduced the biofilm cells with 2 log10 compared to controls. In vivo, a single injection of 10 μg delta-toxin at the place of catheter implantation decreased cells in 1-day old biofilms with 2 log10. The level of reduction was smaller when biofilms were older than 1 day when treated and the effect of delta-toxin disappeared with time. No significant effect was observed in 7-days old biofilms and the delta-toxin effect had disappeared after 7 days. Incubation of 1-day old in vitro biofilms with delta-toxin plus rifampin (10 μg/ml) had more effect (2.50 log10) than each compound alone. This was also true in vivo; delta-toxin plus rifampin (25 mg/kg) gave a 3.81 log10 reduction. This effect also diminished over time and had disappeared after 7 days. Three consecutive doses of delta-toxin (24 hrs interval) reduced the number of biofilm-bacteria 3.5 log10 to 2.03 log10 CFU/catheter at 24 hrs after the 3rd injection. The effect again completely disappeared after 7 days. Three consecutive doses of delta-toxin plus rifampin reduced the number of bacteria 4.81 log10 to 0.69 log10 CFU/catheter. However, no regrowth was observed after 7 or 10 days.

**Conclusion:** We conclude that delta-toxin causes detachment and increases susceptibility to antibiotics. Three consecutive treatments with delta-toxin plus rifampin succeeded in sterilising the FBI.

**P1805** Staphylococcus aureus biofilm formation depends on the S. aureus lineage

**S. Croes**, R. Deuvenberg, M.L. Boumans, P. Beisser, E. Stobberingh, C. Neef (Maastricht, NL)

**Objective:** The aim of the present study was to examine the contribution of the genetic background of both MRSA and MSSA to biofilm formation under physiologic glucose concentration (0.1%).

**Methods:** In vitro biofilm formation of 228 clinical *Staphylococcus aureus* commensal isolates of distinct clonal lineages was characterised by the polystyrene crystal violet adherence assay. Additionally, 26 MSSA isolates recovered from blood from individual patients and associated with either MLST CC8 or CC7 (one of the main clonal lineages among blood stream isolates in our hospital) were tested. These isolates were considered as invasive strains. The genetic backgrounds were determined by spa typing. The associated multilocus sequence typing (MLST) clonal complexes (CCs) were allocated through the SpaServer, since it has been shown that spa typing/ based upon repeat pattern (BURP) results are in agreement with results obtained by MLST. Congo red agar (CRA) screening was used as phenotypic detection of slime producing ability. Furthermore, the accessory gene regulator (agr) types were determined by a real-time multiplex PCR assay.

**Results:** All strains classified as strong biofilm producers, MRSA and MSSA associated with MLST CC8 produced markedly more biomass under all tested glucose concentrations, i.e. 0%, 0.1%, 0.25% and 0.5%. At 0.1% glucose, more than 60% of the *S. aureus* strains associated with MLST CC8 produced thick biofilms, compared to 0–7% for various other clonal lineages. Strong biofilm formation was not related with slime formation, based on CRA screening. Additionally, *S. aureus* bloodstream isolates associated with MLST CC8 and CC7 had similar biofilm forming capacities as their commensal counterparts.

**Conclusions:** Biofilm formation of *S. aureus* on polystyrene surfaces under physiologic glucose concentration (0.1%) was dependent on the clonal lineage. The isolation site was not an (additional) predisposing factor for strong biofilm formation of *S. aureus* isolates associated with MLST CC8 or CC7. CRA screening forms no alternative for crystal violet staining to detect biofilm formation. Furthermore, strong biofilm formation could not be attributed to a specific agr genotype. The agr genotypes were strictly associated with the clonal lineages.

**P1806** Agr-functionality and ability to form biofilm in COL and NRS149 Staphylococcus aureus

**V. Cafiso**, T. Bertuccio, S. Purrello, D. Spina, S. Chiarenza, G. Vizzari, S. Stefani (Catania, IT)

**Objectives:** Sessile communities, known as biofilm, represent the microbial lifestyle responsible for the chronic-polymer-associated infections caused by *Staphylococcus aureus*. The switch from planktonic to sessile is due to the expression of genes involved in the initial attachment and maturation of biofilm. These events are modulated by
Biofilms

a complex network of regulator-systems (agr-locus or master regulators such as sarA) conditioned by environmental variables such as pH, nutrient availability, O2-gradient and cellular-density. We investigated the different levels of expression of four genes involved in biofilm formation (sarA, ralIII, atl and icaA) in two isolates (COL, NRS149 – kindly supplied by NARSNA) of agr-I and II, showing different abilities to form biofilm, in particular, weak and strong.

Methods: Real time RT-PCR was performed using mRNA extracted using time-course experiments (exponential and post-exponential growth-phases) to obtain a relative and comparative quantification of sarA, ralIII, atl and icaA mRNA.

Results: Our results show that the expression of sarA and ralIII regulatory genes was higher during the exponential phase with respect to the post-exponential one in both strains and, in particular, COL presented a greater amount of mRNA transcripts than NRS149. In the same strains the expression of atl (initial attachment gene) and icaD (biofilm accumulation genes) showed an opposite transcription profile, in fact, in COL it was higher in the post-exponential phase while in NRS 149 it was higher in the exponential-phase. Moreover, icaD mRNA was more abundant in NRS149 than in COL.

Conclusions: Our data emphasize the hypothesis of a different functionality of agr-II with respect to agr-I that could be correlated to the diverse abilities to form biofilm of the two agr-groups. agr-I, a weak biofilm producer, in fact, showed a poor expression of the initial attachment and biofilm maturation genes that increased only in the late growth phase, while agr-II, a strong biofilm producer, presented a high expression of the same genes already in the early growth phase.

P1807

Biofilm formation and combinations of virulence factors among methicillin-resistant Staphylococcus aureus isolates in a teaching hospital in Slovenia

V. Ursic*, V. Tomic (Golnik, SI)

Objectives: Staphylococci have been confirmed to form biofilms on various biomaterials. The purpose of this study was to investigate biofilm formation among methicillin-resistant Staphylococcus aureus (MRSA) in a teaching hospital in Slovenia and to assess the relationship between biofilm-forming capacities and virulence determinants/clinical background.

Methods: A total of 105, randomly chosen, non-copy coagulase positive staphylococcal strains, recovered from diverse clinical samples over an 8-year period from 1999 through 2007 were studied. We used the in vitro microtiter plate assay to quantify biofilm formation. We then investigated the presence of several virulence determinants by polymerase chain reaction.

Results: Six determinants (hla, hib, fnbA, clfA, icaA, and agrII) were found to be predominant among these isolates. Enhanced biofilm formation was confirmed in hla-, hib- and fnbA-positive MRSA isolates, both individually and in combination.

Conclusion: Upon review of the associated medical record, we concluded that the biofilm-forming capacities of MRSA isolates from catheter-related cases were significantly greater than those from catheter-unrelated cases. The percentage of hla-, hib-, and fnbA-positive isolates was higher among MRSA isolates from catheter-related cases than those from catheter-unrelated cases. Our studies suggest that MRSA colonisation and infection may be promoted by hla, hib, and fnbA gene products.

P1808

Association of accessory gene regulator locus with biofilm formation and methicillin-resistance in Staphylococcus aureus

A. Ikonomidou*, A. Tsakris, A. Vasieli, S. Xytsas, K. Malizos, S. Pournaras, A. Maniatis (Larissa, Athens, GR)

Objectives: Pathogenicity of Staphylococcus aureus is coordinated by the accessory gene regulator (agr) system. Previous studies indicate that agr group II methicillin-resistant S. aureus (MRSA) may be related to overproduction of biofilm and reduced responsiveness to vancomycin.

The current study investigated the distribution of agr groups among MRSA and methicillin-susceptible S. aureus (MSSA) as well as their association with biofilm formation, in a hospital environment that experiences endemic occurrence of MRSA.

Methods: Forty-two MRSA and 32 MSSA non-repetitive isolates recovered from clinical infections in a Greek university hospital were tested. The presence and the type of the agr locus were determined by PCR and restriction enzyme analysis. Quantitative determination of biofilm formation was performed using a reference microtitre assay. Results were statistically compared to detect the association of agr groups with methicillin resistance and biofilm formation.

Results: agr groups I, II and IV were equally distributed among MRSA and MSSA populations, while agr group III was not detected in MRSA or MSSA. agr group II MRSA isolates showed significantly higher levels of biofilm production in comparison with MSSA isolates of the remaining agr groups as well as with all three agr groups of MSSA isolates. Levels of biofilm production were independent of agr group in MSSA isolates.

Conclusion: The present findings suggest that in our S. aureus population agr group II is simultaneously associated with both biofilm overproduction and methicillin resistance. This indicates another infectious potential of isolates carrying this agr polymorphism.

P1809

Antibacterial activity of ozonised water and gaseous O3 against biofilm


Objectives: Ozone antimicrobial activity is generally known, but its activity against biofilm remains not widely investigated. Specially developed apparatus which allows ozone in statu nascendi production for destruction of bacterial biofilm.

Methods: 18 bacterial strains were cultivated in Luria-Bertani (LB) medium placed into microplate wells and incubated in 37°C for up to 72 h. Planctonic cells were removed and bacterial biofilm layer was treated with freshly obtained ozonised water or gaseous O3. After various contact time of bacteria and ozone solution: from 30 sec to 4 min, and gaseous O3: 20 and 40 min, microplate wells were washed to remove ozone. Alive bacterial cells were stained by PCR and restriction enzyme analysis. Quantitative determination of biofilm were in statu nascendi production from oxygen and preparation of ozonised water, has been applied in this study.

Results: The aim of this study was to analyse bactericidal activity of ozonised water and gaseous O3 against selected clinical strains of Staphylococcus aureus and Pseudomonas aeruginosa, grown in form of biofilms on microplates.

Methods: 18 bacterial strains were cultivated in Luria-Bertani (LB) medium placed into microplate wells and incubated in 37°C for up to 72 h. Planctonic cells were removed and bacterial biofilm layer was treated with freshly obtained ozonised water or gaseous O3. After various contact time of bacteria and ozone solution: from 30 sec to 4 min, and gaseous O3: 20 and 40 min, microplate wells were washed to remove ozone. Alive bacterial cells were stained with 3-(4,5-dimethyl-2-thiazolyl)-2,5 diphenyl-2H-tetrazolium bromide (MTT). Parallelly, appropriate bacterial biofilms not treated with ozone preparations were used as a control. After 2 h of staining, solution was removed and biofilm was solubilised by DMSO/glycine buffer treatment. Solution absorbance was measured at 554 nm. The ozone concentration in ozonised water was determined by iodometric titration using 0.02 mol/L solution of sodium thiosulphate. Concentration of ozone determined chemically, which varied in the range: 1.2–3.6 μg/mL, was compared with antibacterial activity of ozonised water.

Results: Biofilm of P. aeruginosa strains was formed earlier and more intensively as compared with biofilm of S. aureus. Different age biofilms: 2 h (only P. aeruginosa strains) and 24 h, 48 h and 72 h (both groups of strains), were treated by ozone preparations, in order to determine sensitivity to this agent. Some variances between strains were noticed. Ozonised water, produced by prototype apparatus, proved to be very effective biocidal agent toward bacterial biofilm, even after 30 sec of contact. However, gaseous O3 was much less effective as biocidal agent. Even after 40 min of treatment, levels of alive S. aureus and P. aeruginosa biofilm cells were still considerable high.

Conclusion: Ozonised water as effective biocidal agent might be applied for destruction of bacterial biofilm.
Antimicrobial activity of crude eucalyptus oil against Staphylococcus aureus, MRSA, Escherichia coli, Pseudomonas aeruginosa and Candida albicans grown in planktonic and biofilm cultures


Objectives: To investigate the antimicrobial efficacy of crude eucalyptus oil (EO) and determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against a range of microorganisms associated with healthcare associated infections (HAI), when cultured in planktonic and biofilm modes of growth.

Methods: Optimum biofilms were established in sterile flat bottom microplates following 48 hour growth in Muller Hinton broth with Sabouraud dextrose broth used for C. albicans. Biofilm production was verified for Staphylococcus aureus ATCC 6538, meticillin resistant Staphylococcus aureus (MRSA) N315, Escherichia coli NCTC 10418, Pseudomonas aeruginosa ATCC 15442 and Candida albicans ATCC 76615, using Congo red agar. Planktonic and 48 hour biofilm cultures of the five microorganisms were exposed to EO dissolved in Tween 80 (512-0.25 mg/ml) in sterile round bottom and flat bottom microplates respectively. In line with CLSI (formerly NCCLS) guidelines, the MIC and MBC/vancomycin for each microorganism.

Results: Eucalyptus oil demonstrated a broad range of antimicrobial activity, however it was significantly more active against microorganisms grown in planktonic culture, compared with biofilm (P < 0.05). The MIC/MBC (mg/ml) for EO against planktonic cultures were: S. aureus 4/8; MRSA 2/2; E. coli 8/8; P. aeruginosa 256/256; C. albicans 8/32. MIC/MBC (mg/ml) for EO against biofilms were: S. aureus 256/512; MRSA 512/512; E. coli 16/256; P. aeruginosa >512>512; C. albicans 8/32.

Conclusion: Crude EO possessed a broad spectrum of antimicrobial activity against a range of microorganisms cultured in planktonic and biofilm modes of growth. EO may have a place in the clinical setting as an adjunct to, or in combination with currently used disinfectants and antisepsics in the prevention of HAI. Further studies are warranted.

P1811 Activity of oritavancin against a clinical isolate of Staphylococcus aureus in vitro biofilm models

A. Belle,* E. Neesham-Grenon, G. James, E. Pulcini, L. Boegli, T. Parr Jr, M. Moeck (Saint-Laurent, CA; Bozenm, US)

Objectives: Oritavancin (ORI) is a semi-synthetic lipoglycopeptide that is currently in clinical development for serious Gram-positive infections. The activity of ORI and comparator agents was determined against in vitro biofilms derived from a methicillin-resistant Staphylococcus aureus (MRSA) clinical isolate in two biofilm model systems.

Methods: A clinical isolate of MRSA isolated from an osteomyelitis (Birmingham, UK) was used to inoculate 24-h old biofilms on hydroxyapatite-coated glass slides or in MBEC™ Physiology & Genetics Assay plates (Innovotech, Canada). Briefly, 24h old culture of S. aureus (106 CFU/ml). The bottle containing the culture suspension was connected to the BSD through an automatic pump. The suspension was delivered to the BSD at 30 ml/h for 2h and free cells were removed by passing plain RPMI at the same rate for 2h. The biofilms on the catheter segments were kept under continuous flow of fresh RPMI at 10 ml/h. VOZ was tested against 2, 5 and 10 day-old biofilms at initial concentration of 3 µg/ml (similar to its in vivo Cmax). The bottle containing the drug in RPMI was connected to the BSD via a pump and to another bottle containing plain RPMI via a second pump. The rate of delivery of both the drug and the plain medium was kept at 10 ml/h and by this way the drug delivered to the biofilms was exponentially diluted at a rate that follows first order kinetics. Four other doses of the drugs were added to the bottle every 12h (t1/2 of VOZ = 6.5h). Effluent samples from the BSD were taken for cell count (shed cells) at 12 h time interval after each dose.

Results: Our data show that the log10 reductions in the shed cells compared to drug-free control were 0.430, 0.942, 1.0, 1.22 and 1.1.22 for 2 days old, 0.50, 0.98, 0.99, 1.11, and 1.08 for 5 days old, and 0.166, 0.519, 0.0.91, 0.102, and 0.90 for the 10 days old biofilms.

Conclusion: So it is obvious from our model that VOZ was able to minimise but not to stop dispersion of CA cells from the biofilms.

P1812 Effects of voriconazole on the shedding of Candida albicans cells from mature biofilms in an in vitro pharmacokinetic model by using biofilm sampling device

M. El-Azizi, N. Kharalot* (Cairo, EG; Springfield, US)

Objectives: Shed cells from the biofilm may enter the circulation causing serious and very hard to treat biofilm-associated infections. Voriconazole (VOZ) was tested at exponentially decreasing concentrations against mature biofilms of Candida albicans on vascular catheter segments in an in vitro pharmacokinetic model by using biofilm sampling device (BSD).

Methods: Briefly, 24h old culture of C. albicans on Sabouraud agar was used to inoculate RPMI medium to give initial inoculum 1 to 5 ×10⁶ CFU/ml. The bottle containing the culture suspension was connected to the BSD through an automatic pump. The suspension was delivered to the BSD at 30 ml/h for 2h and free cells were removed by passing plain RPMI at the same rate for 2h. The biofilms on the catheter segments were kept under continuous flow of fresh RPMI at 10 ml/h. VOZ was tested against 2, 5 and 10 day-old biofilms at initial concentration of 3 µg/ml (similar to its in vivo Cmax). The bottle containing the drug in RPMI was connected to the BSD via a pump and to another bottle containing plain RPMI via a second pump. The rate of delivery of both the drug and the plain medium was kept at 10 ml/h and by this way the drug delivered to the biofilms was exponentially diluted at a rate that follows first order kinetics. Four other doses of the drugs were added to the bottle every 12h (t1/2 of VOZ = 6.5h). Effluent samples from the BSD were taken for cell count (shed cells) at 12 h time interval after each dose.

Results: Our data show that the log10 reductions in the shed cells compared to drug-free control were 0.430, 0.942, 1.0, 1.22 and 1.1.22 for 2 days old, 0.50, 0.98, 0.99, 1.11, and 1.08 for 5 days old, and 0.166, 0.519, 0.0.91, 0.102, and 0.90 for the 10 days old biofilms.

Conclusion: So it is obvious from our model that VOZ was able to minimise but not to stop dispersion of CA cells from the biofilms.
Evaluation of triazole-echinocandin interactions against Candida parapsilosis planktonic cells and biofilms
A. Chatzimoschou*, M. Simitopoulou, C. Antachopoulos, T.J. Walsh, E. Roilides (Thessaloniki, GR; Bethesda, US)

Objectives: Candida parapsilosis (CP) frequently causes nosocomial bloodstream infections, especially in neonates and patients with central venous catheters. Antifungal triazoles are commonly used in combination with echinocandins in cases of serious fungal infections. While triazoles have been previously shown to have antifungal activity against Candida biofilms (BF), CLSM showed that BF individually treated with an azole, CsA or TCR were morphologically similar to untreated controls. Among the combinations tested, FLC (10 or 125 mg/l) + CsA (0.5 mg/l) displayed the most prominent effects, where shorter hyphae and looser matrix were observed. Similar changes, but to a lesser extent, were evident with VRC (1 or 32 mg/l) + CsA (0.5 mg/l) and PSC (1 or 32 mg/l) + CsA (0.5 mg/l). TCR did not appear to cause any morphological changes, when combined with each of the azoles. FLC (1 and 8 mg/l) + CsA (0–32 mg/l) showed increasing BF damage (16–40%) with increasing CsA concentrations, as compared to drugs alone (FLC 7–10% and CsA 9–14%; n = 4).

Results: Against PL, a synergistic interaction was observed between 32–128 mg/L of PSC combined with 0.008–0.25 mg/L of CAS. By contrast, antagonism was observed when either of the two triazoles was combined with AND at the following concentration ranges: 128–1024 mg/L PSC + 0.03–0.5 mg/L AND, 16–512 mg/L VRC + 0.008–0.015 mg/L AND or 128–512 mg/L VRC + 0.03–0.25 mg/L AND. Against BF, all drug combinations demonstrated indifferent interactions.

Conclusions: The differential interactions of synergy or antagonism between triazoles and echinocandins observed against CA PL become indifferent in the presence of CA BF.

Combined activities of antifungal azoles with calcineurin inhibitors against Candida albicans biofilms

Objectives: Candida albicans (CA) biofilms (BF) are frequent causes of foreign body-related infections and are resistant to even high concentrations of azoles (Katragkou et al, AAC 2008; 52: 357). Calcineurin inhibitors, such as cyclosporine A (CsA) and tacrolimus (TAC), are synergistic with fluconazole (FLC) against CA planktonic cells. We aimed to determine the efficacy of FLC, voriconazole (VRC) or posaconazole (PSC) combined with CsA or TCR against CA BF.

Methods: CA-M61, a clinical BF-producing strain was used. Confocal scanning microscopy (CLSM) and XTT assays were performed. For CLSM, BF were formed on silicone disks placed in 12-well plates at 37°C under continuous rocking for 48 h. BF were then incubated with either no drugs, or with FLC (10 and 125 mg/l), VRC (1 and 32 mg/l), PSC (1 and 32 mg/l), CsA (0.5 mg/l), TCR (20 mg/l) alone, or with each of the azoles combined with CsA or TCR for 24 h. Treated BF were stained with FUN-1 and concanavalin A-Alexa Fluor 488 conjugate. Stained BF were examined by CLSM. Drug interactions were also assessed by checkerboard micromethod and XTT metabolic assay. For these experiments, BF were grown in 96-well plates at 37°C for 24 h and were then incubated with fourfold dilutions of each azole (0.06–1000 mg/l) combined with CsA or TCR (0.016–64 mg/l) for 24 h. CLSM and XTT assays were performed 2 and 5 times, respectively.

Results: Synergy, antagonism or indifference was concluded when the observed BF damage was significantly higher than, lower than or equal to the expected damage, respectively.

Conclusions: Among the combinations tested, FLC+CsA demonstrated a collaborative effect against CA BF followed by VRC+CsA and PSC+CsA. Combination antifungal treatment mediated via different mechanisms may have utility against CA BF. Combination therapy may prove a novel therapeutic intervention in difficult to treat BF-related infections.
**P1817** A scanning electron microscopy based quantitative method to evaluate plaque accumulation in patients undergoing different oral home care protocols

**F. Tessarolo**, E. Bressan, C. Tomasi, F. Piccoli, G. Nollo, I. Caola (Trento, Padua, IT, Gothenburg, SE)

**Objectives:** The quantification of plaque biofilm (PB) in oral cavity surfaces is an important indicator to test the efficacy of oral home care protocols. The study aimed at defining a quantitative method for evaluating PB formation by scanning electron microscopy (SEM) on titanium surfaces in patients rehabilitated by osseointegrated implants. The feasibility of the method was verified by running a pilot study to evaluate the effect of antiseptic in preventing plaque accumulation on healing abutments (HAs).

**Results:** Antibiotic-loaded specimens released high and inhibitory concentrations of G and V and V alone in PMMA cement reduced the bacterial adhesion of susceptible and intermediate-resistant *Staphylococcus* strains (no bacterial growth); only low drug concentrations from the combination determined delayed and poor bacterial colonisation. The amount of antibiotic released exerts different inhibitory capacity on bacterial growth and adhesion, being the effect strain-dependent. The higher release of drug from PMMA resulted in a stronger and more prolonged inhibition of bacterial growth.

**Conclusions:** (i) The presence of G in PMMA specimens reduces the bacterial adhesion in susceptible and intermediate-resistant *Staphylococci*. (ii) The combination G-V exhibits synergistic activity against all strains; moreover, inhibits the growth and adhesion of V-resistant strains. (iii) The anti-adhesive effect of the antibiotic-loaded cement depends on the characteristics of the microorganism and its capacity of adhering to antibiotic-loaded surfaces. (iv) Bacterial adhesion is reduced in specimens presenting a higher capacity of antibiotic elution.

**Figure 1.** Plaque on healing abutment imaged by SEM in BSE mode: (a) raw and (b) thresholded image. (c) Bacterial morphologies in plaque biofilms at higher magnification.

**Methods:** The study was designed as a single blind randomised crossover controlled experiment to reveal the influence of 0.12% CHX mouthrinses in the formation of PB during a 7-days plaque accumulation model. Ten new HAs were placed in 5 voluntary patients one week after implant surgery and removed after 7 days. At removal, a new set of HAs was placed and removed one week after. During the two testing period, patients were instructed to apply the following protocols in a randomised order: CHX mouthrinsing twice daily and no brushing (Test); no CHX mouthrinsing and no brushing (Control). HAs were fixed immediately after removal in 2.5% glutaraldehyde phosphate-buffered solution, washed in buffer, dehydrated by graded alcohol series, vacuum dried, and gold sputtered. One low-magnification image per sample of the coronal surface was acquired by SEM in backscattered mode and then thresholded by an automated image analysis software. PB amount was computed by considering dark pixels associated to PB and bright pixels representing the clean surface of the HA. Mann-Whitney test was used to compare groups.

**Results:** The protocol for preparing and observing PB by SEM provided quantitative data with a wide variation among subjects and implant sites. The mean values of the ratio of titanium surface covered by PB were 23% (S.E. 34) and 33% (S.E. 37) for test and control respectively, but no statistically significant difference was detected between the groups.

**Conclusion:** The quantification of PB on HAs by SEM was feasible and allowed to create a non-subjective indicator of PB amount. HA is a valid substrata for oral microbiota adhesion and growth, easy to remove and substitute without causing any trauma to peri-implant tissues. The sensitivity of the presented quantification method and the inter-patients and inter-implant-sites variability demands for a larger number of samples to have higher statistical confidence on the results obtained in this pilot study.

**P1818** Evaluation of natural compounds derived from plants on planktonic and sessile form of *staphylococci*


**Objective:** In this study the activity of different compounds of natural origin on planktonic and sessile cultures of *S. aureus* and *S. epidermidis* was tested. We used: i) a synthetic derivative of the dihydroxybenzofuran (DHBF), originally isolated from *Krameria lappacea*; ii) two quaternary benzo[c]phenanthridine alkaloids (QBAs), sanguinarine (SA) and chelerythrine (CH), extracted from *Papaveraceae* and *Fumariaceae*; iii) proanthocyanidin (proAC) and a standardised extract prepared from *Vaccinium myrtillus* L (BB). The presence of buffer in planktonic cultures, both proAC and BB do not show an antibacterial activity in the range of used concentration, while DHBF and both QBAs show a clear bacteriostatic and/or bactericidal activity on the two tested strains. Interestingly, inhibition of biofilm formation was observed for all tested compounds, including proAC and BB. Moreover, DHBF, proAC and SA reduced *S. epidermidis* preformed biofilm despite the active concentration required are quite high, SDS-PAGE and zymogram analyses revealed diverse modification of surface protein pattern for treated bacteria in comparison with that of untreated bacteria, depending on the specific molecule used. In particular, the protein pattern of DHBF-treated samples is similar to the untreated samples. Furthermore, the modification profile observed for each strain is similar for both polyphenols (proAC and BB) and QBAs.

**Conclusion:** Our data suggest that some of the analyzed compounds could be proposed as antibacterial drugs in infection sustained by *Staphylococcus* spp. Their action on sessile phenotype renders them
Molecular bacteriology – part 3

The role of CTX phage in the emergence of the Vibrio cholerae variants with different genomic organisations

B. Bakhshi*, M.R. Foursafie (Tehran, IR)

Objectives: Cholera toxin is encoded by the ctxAB operon in the genome of a filamentous bacteriophage (CTXphage). The multiple copies of CTX prophage are tandemly arranged in El Tor strains of V. cholerae but the number and arrangement of the CTX elements and the repetitive sequences are known to vary in different toxigenic strains and is a useful basis for study of diversity and characterisation of isolates.

The objective was to investigate the genetic arrangement and copy number of CTX elements in the V. cholerae strains isolated during outbreaks in Iran.

Methods: Extracted DNA of 37 isolates was subjected to digestion with PsiI restriction enzyme which has only one cutting site inside the CTX element. The fragments were separated with agarose gel electrophoresis and then transferred to positively charged nylon membrane. Digoxigenin-labeled ctxA and zot gene probes were used for hybridisation.

Results: The results showed 3 hybridisation profiles for each of the probes used with the bands ranging from 4.2 to 8.3 kb in size. The results showed that CTX element in 30% of isolates was residing on the fragments of 5.6 kb (profile A) when either ctx or zot probe was used. The strains with hybridisation profile C showed two fragments of 6.9 and 8.3 kb with either ctx or zot probes for 17% of the isolates. In the strains with hybridisation profile B, fragments of 4.2, 5.6 and 6.9 kb was displayed when zot probe was used. When the same strains were hybridised with ctx probe, only one band of 6.9 kb was detected.

Conclusion: Southern blot hybridisation using ctx or zot probes showed the presence of 1 (30%), 2 (17%) and 3 (53%) copy numbers among the isolates. The isolates with 1 or 2 copies were shown to carry the whole CTX element with 6.9 kb. On the hand, 2 of the CTX elements (5.6 kb) were found to be truncated in ctxAB for the isolates with 3 copies of CTX element. These variations in the CTX phage acquisition results in the emergence of new variants of V. cholerae with different toxicity power.

The influence of enterocin B on the morphophysiology of Escherichia coli

O. Rybalchenko, E. Yermolenko, A. Kolobov, A. Chernish, A. Svorcov*, A. Totolam (St. Petersburg, RU)

Objectives: Bacteriocins are the novel target for the search of new antimicrobial agents different from antibiotics. Previously it was shown that enterococcal bacteriocin (enterocin B) is highly effective against Gram-positive bacteria (Moreno et al. 2006, Nilson et al.1998, Casaus et al. 1997). However, the activity of this enterocin against Gram-negative bacteria never been described. The aim of this study was to reveal peculiarities of action of enterocin B on the growth and morphology of Escherichia coli.

Methods: Enterocin B (B5) and its analog (B2) without the several amino acids at the N-terminus, were synthesized. The peptides in concentrations (0, 02–0, 0002mg/ml) were added on the surface of the solid medium (Tryptone agar) immediately after inoculation of the culture of E. coli ML-35p at the exponential phase of growth (6 lg CFU/ml). The lysozyme (50 mcg/ml) was used as a control. After incubation for 24 h the diameters of inhibition growth zones have been measured. The thin sections taken from the marginal areas of inhibition growth have been studied by electron microscopy.

Results: Both synthetic peptides and lysozyme showed inhibitory activity against culture of E. coli. Diameters of inhibition of the growth zones on the lawn of indicator bacteria were 12, 12 and 14 mm, consequently. The factors under study caused different damages of E. coli cells. Peptides B5 and B2 used in concentration 0.002 mg/ml caused pathological changes approximately 20% of the cells of indicator culture.

Under electron microscopy we could determine rejection of the cell wall from the cytoplasmic membrane of bacteria. Most likely the destruction of the cell wall was caused by the pressure from the inside. One of the possible mechanisms explaining this effect is the action on the membrane of Gram-negative bacterial cell as it was earlier described regarding Gram-positive bacteria.

Conclusion: Enterocin B can inhibit the growth of E. coli and cause a damage of surface structures of these bacteria. This fact is important for the possible application of enterocin B and it analogs for the therapy and prophylactic of infections, caused by E. coli and other Gram-negative bacteria. The work was supported by grant of RFIF 06–04–48949.

Complementation in hypermutable Escherichia coli strains shows that mismatch repair and 8-oxoG pathway genes are not always responsible for mutator phenotype


Objective: Few published data have confirmed the role of genes belonging to mismatch repair system (MMR) or 8-oxoG pathway (GO) in the mutator phenotype (MP) by complementation studies in bacteria. The aim of our study was to characterise the genes involved in the MP in four Escherichia coli isolates from different origins.

Methods: PCR-amplification of the mismatch repair genes (mutS, mutL, mutH, uvrD) and 8-oxoG pathways (mutT, mutY) from E. coli MG1655 was performed, and these genes were cloned in the multicopy plasmid pGEM-Easy and transformed into four hypermutable strains: ECU24 and DA6879 urine isolates, ECC19 recovered from faeces from a healthy volunteer and ECB23 from a surgical wound in an immunocompromised patient. These wild-type genes were also transformed in a normomutator laboratory strain, as control. Mutation frequency was calculated in wild-type and transformed isolates.

Results: Mutation frequency decreased when wild-type mutS gene was hyper-expressed in ECU24 (81x reduction), DA6879 (x152) and ECB23 (x225) strains, suggesting that mutS gene was defective. It was demonstrated by sequencing, so the ECU24 strain presented a deletion of 8 bp in mutS altering the open reading frame; DA6879 strain carried a deletion of 11,903 bp including mutS and rpoS genes; and in ECB23 strain a copy of IS10 in the position 2067 of the mutS gene (coding for the ATP binding domain) was detected. The ECC19 strain showed the highest mutation frequency value (500-fold higher than modal value for E. coli). Unexpectedly, the ECC19 mutator phenotype was not complemented with any of the mut genes studied, suggesting that this strain was not defective in either MMR or GO pathways. Interestingly, a partial restoration of mutation frequencies (5–10x reduction) was obtained in ECU24 by hyper-expression of the mutH gene, or by mutT gene in ECB23 and DA6879 strains, while this phenomenon was not observed in the control strain.

Conclusions: A defective mutS gene was responsible of the MP in three out of four strong hypermutable E. coli strains, similar to described in Pseudomonas aeruginosa. In one strain, and as it has been shown in Salmonella typhimurium (Yang B, 2008) the MMR or GO systems do not appear involved in some cases of MP. In strains with defective mutS gene, the hyper-expression of mutL or mutT genes could partially revert the MP, suggesting that a second order of selection by hitchhiking process can be possible.

Comparative distribution of phylogenetic groups, virulence genes and antimicrobial resistance in Escherichia coli isolated from blood, urine and faeces


Objectives: We compared the distribution of phylogenetic groups and virulence genes of Escherichia coli between pathogenic strains (from
blood and urine) and commensal strains (from faeces), and antimicrobial resistance of *E. coli* isolated from blood and urine.

**Methods:** A total of 550 non-duplicate *E. coli* isolates (145 from blood, 200 from urine, and 205 from faecal specimens of healthy humans) were consecutively obtained. PCR experiments for phylogenetic groups (A, B1, B2, D) and nine virulence genes (sfa1, sfg, hlyA, cnf1, iutA, fyuA, iroN, traT, PAI) were performed by using published primers for all isolates. Antimicrobial susceptibility tests for ampicillin, piperacillin, amoxicillin/clavulanic acid, pipercillin/tazobactam, cephalazin, cefoxitin, cefotaxin, ceftazidime, cefepime, amikacin, gentamicin, tobramycin, tetracycline, ciprofloxacin, trimethoprim/sulfamethoxazole, imipenem were determined by VITEK 2 automated system (bioMérieux, VITEK) and ESBL confirmatory test was performed according to the CLSI guideline for 345 *E. coli* isolates from blood and urine. Statistical analyses were performed by using chi-square tests. A P value of <0.05 was considered statistically significant.

**Results:** The phylogenetic distribution showed similar pattern between *E. coli* from blood and urine (B2 (44.8%, 58.5%, respectively) > D (29.0%, 23.0%, respectively) > A (18.6%, 9.5%, respectively) > B1 (7.6% and 9.0%, respectively). However, isolates from faeces revealed different distribution (A (38.0%) > B2 (22.9%) > D (21.0%) > B1 (18.0%).

Out of the nine virulence genes, the prevalence of all but sfa1 were significantly higher in pathogenic strains than in commensal strains, and that of PAI, fyuA and traT were significantly higher in *E. coli* from blood than isolates from urine.

The antimicrobial resistance rate showed no significant difference between in *E. coli* from blood and *E. coli* from urine, but in phylogenetic group B2, the prevalence of ESBL (18.5% vs 6.0%) was significantly higher in *E. coli* from blood than in *E. coli* from urine.

**Conclusion:** Most (78.3%) of pathogenic *E. coli* strains belonged to the phylogenetic groups B2 or D. Unexpectedly, as many as 43.9% of commensal strains belonged to group B2 and D.

The finding that the prevalence of PAI, fyuA and traT were significantly higher in *E. coli* from blood than *E. coli* from urine suggests the presence of these virulence factors indicate higher risk of bacteremia.

**P1823** Enteric pathotypes of *Escherichia coli* involved in infectious diarrhoeal syndrome


**Objectives:** 1. Assessment of incidence for enteric pathogenic strains of *E. coli* in a samples of patients with acute diarrhoeal syndrome (ADS), with or without concomitant HIV infection. 2. Assessment of the role of classical diagnostic versus genotypical methods, in diagnosing ADS caused by *E. coli*.

**Methods:** The study included 1830 patients, with ADS, hospitalised in SVB, between 1st Jan 2006–31 June 2007. There were excluded other cases of infectious diarrhoea, except *E. coli*: viruses, parasites, other enteral bacterial pathogens. The classical methodology was based on isolation, phenotypic identification and serological testing. Isolation was made on mild/moderate selective media (MacConkey, SMAC, CLED, Hektoen/ADCL). Phenotypic identification was based on growth culture proprieties on multistests media: TSI, MIU, MILF, Citrate and confirmed by: API 20 E, ID 20 E, VITEK 2 C. Serology was based on antigenity criteria: serotyping OB (Denka Seiken, Tokio, Japan). Microscopic examination, macroscopic aspects and epidemiological data were used for obtaining an oriented stool culture for *E. coli*. Internal quality control was provided by using *E. coli* ATCC 25922. All strains of *E. coli* isolated in pure culture were analyzed through multiplex PCR in INCDMIC.

**Results:** We identified 410 strains of *E. coli*: 369 HIV(+) and 41 HIV(–), the most ones at the age group 0–5 years: 74.3% cases. Enteric pathogenic *E. coli* was identified by phenotypical methods in 12.29% (46 strains) and by molecular methods in 13.12% (54 strains). We identified 31.5% EPEC pathotype, 0.7% EIEC pathotype and 0.5% EHEC pathotype by phenotypical tests. The molecular tests showed 5.12% atypical EPEC (eae gene), 0.7% typical EPEC (bfp and eae genes), 2.7% atypical STEC/VT (with one gene or association of 2–3 genes: eae, stx1, stxII, mdt), 0.2% typical STEC/EHEC (all 4 genes), 4.4% EAE (eae gene), 0% EIEC and ETEC (genetic unconfirmed).

There were no enteric pathogenic strains of *E. coli* isolated from HIV (+) patients.

**Conclusion:** 1. Pathotypes of *E. coli* was confirmed by genotypical methods, only in HIV(+) patients; in HIV(–) patients, ADS occurred by other non-infectious pathophysiologioal mechanisms of SIDA. 2. In diagnosing enteric pathotypes of *E. coli*, a comparative analyse of the two techniques used, lead to superior results for genetic methods versus phenotypical methods (13.12% versus 12.29%).

**P1824** Development and validation of a real-time PCR assay for detection of enteropathogenic *Escherichia coli*, as part of a Dutch study on the epidemiology of gastro-enteritis

M.C. Scholts*, G.J. Wisselink, R.F. de Boer, A.M.D. Koostra-Smid, Y.T.H.P. van Duynhoven (Groningen, Bilthoven, NL)

**Objectives:** Enteropathogenic *Escherichia coli* (EPEC) is a major cause of infantile diarrhoea predominantly in developing countries but are also identified with increasing frequency in developed areas and in adults. Typical and atypical EPEC strains harbour the pathogenic “locus of enterocyte effacement” (LEE) region, which is responsible for the phenotype of attaching-and-effacing lesions. On this region the escV gene is located. Typical EPEC harbour an additional adherence factor plasmid on which the bfpA gene is located. Identification of EPEC strains is currently based on adherence assays and serotyping with specific antisera. Both assays are time-consuming and demand considerable technical expertise.

In May 2008 a study in 6 Dutch hospitals has started to assess the incidence, aetiology and course of patients hospitalised for gastro-enteritis (GEops study). As part of this study, and to facilitate rapid diagnosis, a real-time PCR assay was developed and validated for the detection of typical and atypical EPEC.

**Methods:** A real-time PCR assay targeting the escV and bfpA genes was developed. DNA isolation from stool samples was performed with the easyMAG specific A stool protocol (bioMerieux). As internal control, phocine herpes virus-1 was used. The selectivity of the assay was validated using a panel of well-characterised EPEC isolates (n = 35), a panel of *E. coli* spp. non-EPEC isolates (n = 52) and a panel of non-*E. coli* strains (n = 30). Analytical sensitivity was assessed by dilution series (n = 2), spiked in a pool of faecal matrices, with different consistencies. Also, a clinical validation was performed on stool samples routinely screened for bacterial and parasitic enteric pathogens (n = 101).

**Results:** The assay proved to be specific for EPEC, as no cross reaction was observed. All thirty-five isolates of EPEC scored positive in the real-time PCR for the escV target. Additionally, 9 isolates scored also positive for the bfpA target. The assay is capable of detecting approximately 6600 CFU per gram of stool, for both targets. Typical and atypical EPEC DNA was detected with this real-time PCR assay in respectively 1% and 16% of the 101 clinical samples. PCR inhibition was not observed in these clinical samples.

**Conclusion:** These data prove the assay to be a sensitive method for the detection of typical and atypical EPEC in stool samples. The assay is currently used as a rapid screening tool for the detection of EPEC in the GEops study.

**P1825** Development and validation of a rapid molecular screening panel for detection of enteroaggregative *Escherichia coli*, as part of a Dutch study on the epidemiology of gastro-enteritis

M.C. Scholts*, G.J. Wisselink, R.F. de Boer, A.M.D. Koostra-Smid, Y.T.H.P. van Duynhoven (Groningen, Bilthoven, NL)

**Objectives:** Enteroaggregative *Escherichia coli* (EAEC) is an increasingly recognized enteric pathogen. It causes acute and persistent
Molecular bacteriology – part 3

S529

diarrhoea among children, adults and HIV-infected persons. The Hep2- assay is considered to be the gold standard for the detection of EAEC. However, the Hep2-assay is not suitable as a diagnostic tool, as it is time-consuming and demands considerable technical expertise. In May 2008 a study in 6 Dutch hospitals has started to assess the incidence, aetiology and course of patients hospitalised for gastro-enteritis (GEOops study). As part of this study, and to facilitate rapid diagnosis, a molecular screening panel was developed and validated for the detection of EAEC.

Methods: Since EAEC strains are heterogeneous, real-time PCR assays were developed targeting not only the aat and aggR genes, but also the astA and pie genes. In this study results were considered EAEC positive if aat, aggR or a combination of 2 or more targets were detected. DNA isolation from stool samples was performed with the easyMAG specific A stool protocol (bioMérieux). As internal control, phocine herpes virus-1 was used. Selectivity of the assays was validated with a panel of well characterised EAEC strains (n = 16), a panel of E. coli spp. non-EAEC strains (n = 77) and non-E. coli strains (n = 30). Analytical sensitivity was assessed by dilution series (n = 2), spiked in a pool of faecal matrices, with different consistencies. A clinical validation was performed on stool samples routinely screened for bacterial and parasitic enteric pathogens (n = 101).

Results: Eleven (69%) of the 16 EAEC strains scored positive with the real-time PCR. 5 strains remained negative or were only astA positive. Of the 107 non-EAEC and non-E. coli strains 12 were astA positive. The real-time PCR assays were capable of detecting approximately 3520 CFU per gram of stool for all targets. Totally 31% of the clinical samples scored positive for one or more of the 4 targets. Fourteen (14%) of these clinical samples were suspected for containing EAEC. The remaining 17% scored positive for astA only. PCR inhibition was observed in less than 1% of these clinical samples.

Conclusion: These data prove the molecular screening panel to be a useful tool for the detection of EAEC in stool samples. The assay is currently used as a rapid screening tool for the detection of EAEC in the GEOops study.

P1828

Molecular characterisation of pUO-StVR2 variants belonging to a family of virulence-resistance hybrid plasmids of Salmonella enterica serovar Typhimurium originated from pSLT

A. Herrero, R. Rodicio, P. García, M.C. Mendoza, B. Guerra, M.R. Rodicio ° (Osejo, ES; Berlin, DE)

Objective: To investigate the genetic structure of five variants of pUO-StVR2, a virulence-resistance hybrid plasmid originated from pSLT, the virulence plasmid specific of Salmonella enterica serovar Typhimurium.

Methods: Analysis of the plasmid variants pUO-StVR4 to pUO-StVR8, was performed by PCR amplification, Southern hybridisation and DNA sequencing.

Results: pUO-StVR2 differs from pSLT by a ca. 12 kb deletion and a 47.6 kb insertion of foreign DNA. The latter consists of a central resistance island and two flanking regions. The resistance island is responsible for the ampicillin-cloramphenicol-streptomyacin/spectinomycin-sulphonamycin-tetracycline/blaoxA1-catA1-aadA1-sul1-tet(B) pattern conferred by the plasmid to S. Typhimurium. The left and right flanking regions provide genes for plasmid maintenance and iron acquisition, respectively. pUO-StVR4 shared the tripartite structure of pUO-StVR2, but contained additional DNA inserted within the left region. All other variants lacked the right region, this being the only difference found between pUO-StVR5 and pUO-StVR2. In the remaining variants, the resistance clusters suffered deletions and/or insertions that reduced or expanded the resistance profile. pUO-StVR6 and pUO-StVR7 (ampicillin-streptomycin-sulphonamycin) had different deletions that removed the catA1 and tet(B) genes, which were shown to be carried by Tn10 and Tn10-like transposons, respectively. In addition, pUO-StVR7 has gained a second integrin (1000 bp/tadA22), apart from InH (2000 bp/blaoxA1-aadA1) that, associated to a Tn21-like element, is present in the six members of the pUO-StVR2 family. pUO-StVR8 has acquired a orf513-dfrA10-3CS element, which converts InH into a complex integron, and conferred additional resistance to trimethoprim. The insertion site of the foreign DNA, between the catAB and pefI genes of pSLT, coincide in all members of the group, but the deletion affecting the resistance cluster in pUO-StVR6 has spanned into the pSLT DNA downstream of pefI.

Conclusions: The study reports on new plasmid variants which can be generated in nature through insertion and/or deletion of virulence and/or resistance determinants. Such changes constitute an interesting example of evolutive engineering, and may have important consequences for the interaction of pathogenic bacteria with the human host, leading to more virulent strains and of more difficult treatment.

P1827

Rapid molecular diagnosis of severe sepsis in patients with SIRS

J.C. Palomares °, B. Puche, A. Martos, F. Lucena, M. Marín, E. Martín-Mazuelos (Seville, ES)

Objective: Blood culture (BC) is considered the gold standard for detection of bacterial bloodstream infections, but the aetiology of sepsis is identified only in a small number of patients and results are usually unavailable until next day. We assessed the utility of an automatic method to extract DNA for a multiplex real-time PCR Septifast (SF, Roche Molecular Systems) in rapid diagnosis of bacterial and fungal bloodstream infections.

Methods: A total of 73 adult patients (76 samples) from the intensive care unit (ICU) of the Valme University Hospital with suspected blood-stream infection and at least two criteria of the systemic inflammatory response syndrome (SIRS) were enrolled. Blood samples for BC and SF (three milliliters of whole blood in EDTA bottles) were simultaneously drawn. DNA extraction was performed by an automatic method (MagNAPure Compact. Roche) and compared with the manual method recommended by the manufacturer (Septifast Prep kit). The amplification was performed with the LightCycler® Septifast kit (Roche Molecular Systems). The final diagnosis was adjudicated by 2 independent infectious diseases specialist not aware of the SF results.

Results: Twenty-seven cases got concordant negative results by BC and PCR. Seven cases were positive concordant by BC and PCR (4 S. aureus, 1 Streptococcus pneumoniae, 1 Staphylococcus epidermidis and 1 Pseudomonas aeruginosa + Candida albicans); median time to positivity was 23 h (range 10–48 h). In 7 additional patients with sepsis, BC were false-positive with coagulase-negative staphylococci (contamination). In 3 patients with positive BC (1 Salmonella enteritidis – not included in SF master list, 1 P aeruginosa and 1 Escherichia coli), SF was false-negative. SF detected clinical significant microorganisms in 10 cases, not detected by BC (2 E. coli, 2 S. aureus, 2 Aspergillus fumigatus, 2 S. pneumoniae, 1 P aeruginosa and 1 Klebsiella pneumoniae/oxytoca). 68 patients (93%) had received prior antibiotic therapy.

Conclusions: SF using an automatic DNA extraction method allows the detection and identification of potentially significant bacteria and fungi in 4 hours (2 h 30 min less than the manual one), showing a better sensitivity than BC for the diagnosis of SIRS (23% vs 18%); PCR could serve as an adjunct to current culture methods to facilitate same-day microbiological diagnosis of sepsis.

P1828

Diagnosis of periprosthetic joint infection using multiplex PCR in sonication fluid of removed implants

Y. Achermann °, M. Vogt, M. Leunig, H. Hermann, J. Wüst, A. Trampuz (Baur, Basel, Zurich, CH)

Objective: The microbiological diagnosis of periprosthetic joint infection (PJJ) is crucial for successful outcome. Cultures have limited sensitivity, especially in patients receiving previous antimicrobial treatment. We compared the multiplex real-time PCR test (Septifast, Roche Diagnostics) for detection of microbial DNA with cultures of sonication fluid.

Methods: We prospectively included patients in whom an infected prosthesis (or part of it) was removed from 8/08 through 12/08. PJJ
was defined as visible purulence, acute inflammation on histopathology, sinus tract or microbial growth in periprosthetic tissue (at least 2 positive tissues were required for low-virulent organisms). The removed implant was sonicated (described in NEJM 2007;357:654) and the resulting sonication fluid was cultured aerobically and anaerobically. In addition, 1 ml of the fluid was investigated using SeptiFast.

**Results:** In this ongoing study, 21 episodes of PJI in 18 patients were included (median age 75 y; range 49–86 y), including hip (n = 9), knee (n = 9), shoulder (n = 2) and ankle prosthesis (n = 1). The following pathogens were cultured: Staphylococcus aureus (n = 3), coagulase-negative staphylococci (n = 6), streptococcus agalactiae (n = 1), Propionibacterium acnes (n = 2) and mixed infection (n = 3). In sonication culture, the causative organism was identified in 14 (67%) cases and by SeptiFast PCR in 18 (86%) cases. In 7 false-negative cultures, the pathogen was identified only by SeptiFast (4 S. aureus, 1 coagulase-negative staphylococcus, 1 streptococcus sp.) or an additional microorganism was found with the SeptiFast (Klebsiella oxytoca/pneumoniae). In all 3 false-negative cases by SeptiFast, P. acnes was missed. All patients with false-negative cultures received previous antibiotic therapy. Among 11 cases receiving antibiotics for a median of 16 d (range 3–60 d) before the diagnostic procedure, SeptiFast was positive in all 11 (100%), whereas sonication cultures grew the organism in only 4 (36%).

**Conclusion:** SeptiFast in sonication fluid has a higher sensitivity for diagnosis of PJI compared to sonication culture (86% vs 67%), particularly among patients receiving previous antibiotic therapy (100% vs 36%). All missed organisms by SeptiFast were P. acnes, which can not be detected due to lack of specific primers in the PCR kit. With modified primer sets, multiplex PCR has the potential for further improvement of the diagnosis of PJI, particularly in patients receiving antibiotics.

**References:**


2. Joly-Guillou ML, Joly-DuBois F, van der Linden B. A total of 1247 samples (249 articular, 73 pericardic, 161 peritoneal and 764 pleural fluids) were investigated by PCR in our molecular diagnostic laboratory between 2000 and 2008. The different home-made molecular tests were 7 real-time TaqMan PCR detecting Chlamydia trachomatis, Neisseria gonorrhoeae, Mycobacterium tuberculosis complex, Borrelia burgdorferi, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, and 2 broad-range PCR with classic amplification of the 16S rDNA followed by sequencing of the amplified product i.e. a bacterial broad-range and a Mycobacterial spp. broad-range PCR.


4. Joly-Guillou ML, Joly-DuBois F, van der Linden B. A total of 1247 samples (249 articular, 73 pericardic, 161 peritoneal and 764 pleural fluids) were investigated by PCR in our molecular diagnostic laboratory between 2000 and 2008. The different home-made molecular tests were 7 real-time TaqMan PCR detecting Chlamydia trachomatis, Neisseria gonorrhoeae, Mycobacterium tuberculosis complex, Borrelia burgdorferi, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, and 2 broad-range PCR with classic amplification of the 16S rDNA followed by sequencing of the amplified product i.e. a bacterial broad-range and a Mycobacterial spp. broad-range PCR.


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For the bacterial broad-range PCR, 2/23 (1 Rothia mucilaginosa, 1 Pseudomonas spp.) were positive both in freshly biopsied specimens and paraffin-embedded adenopathies. For one of the PCR corresponding to 24% (23% and 35% respectively) of the samples. Only one sample contained inhibitors. As many as 34% of the paraffin-embedded samples gave a positive result for M. tuberculosis complex (see Table).

**Results:** On the 377 samples, 483 PCR tests were performed. Ninety-two samples (73 fresh biopsies and 19 paraffin-embedded) were positive for one of the PCR corresponding to 24% (23% and 35% respectively) of the samples. Only one sample contained inhibitors. As many as 34% of the paraffin-embedded samples gave a positive result for M. tuberculosis complex (see Table).

**Conclusion:** Carefully indicated molecular methods could bring a possible added value over conventional testing especially for fastidious organisms. Here for 7% (83 out of 1247) normally sterile body fluids tested by various PCR) a difficult to grow bacteria has been detected and identified by specific PCR or broad-range PCR. The economical and clinical impact of such a diagnosis strategy need to be assessed.

<table>
<thead>
<tr>
<th>Adenopathies</th>
<th>Samples</th>
<th>All (n=377)</th>
<th>All + (n=83)</th>
<th>% of + for M. tuberculosis/total no. of samples tested</th>
<th>% of + for bacterial broad-range/total no. of samples tested</th>
<th>% of + for C. trachomatis/total no. of samples tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural</td>
<td>764</td>
<td>34%</td>
<td>23/699</td>
<td>3%</td>
<td>9/62</td>
<td>1/11</td>
</tr>
<tr>
<td>Pericardial</td>
<td>73</td>
<td>6%</td>
<td>4/67</td>
<td>6%</td>
<td>1/11</td>
<td>0/0</td>
</tr>
<tr>
<td>Pleural</td>
<td>764</td>
<td>34%</td>
<td>23/699</td>
<td>3%</td>
<td>9/62</td>
<td>1/11</td>
</tr>
</tbody>
</table>

For the bacterial broad-range PCR, 2/23 (1 Rothia mucilaginosa, 1 Pseudomonas spp.) were positive both in freshly biopsied specimens.

**Conclusion:** Assessing the performance of molecular diagnosis on clinical management will be the future challenge for molecular diagnosis. These data will help to better focus the indications for molecular tests. Here for 24% of the adenopathies tested, a fastidious pathogen has been detected and identified by specific PCR or bacterial broad-range PCR. Nevertheless more studies with economical and clinical impacts are needed.

**P1832 Is multiplex PCR (SeptiFast) useful for diagnosis of infectious endocarditis?**

A. Conen*, N. Schaub, Y. Achermann, M. Battegay, R. Frei, A. Trampusz (Basel, CH)

**Objectives:** Blood cultures (BC) represent the main diagnostic tool in patients with suspected infectious endocarditis (IE). However, BC can be false-negative when difficult-to-culture microorganisms are involved or patients have received previous antibiotic treatment. The multiplex real-time PCR SeptiFast (Roche Diagnostics) is a culture-independent method that detects microbial DNA of 25 bacterial and fungal pathogens within 6h. We assessed the diagnostic value of SeptiFast in comparison with BC in patients with IE.

**Methods:** We prospectively included adult patients with suspected native or prosthetic valve IE, defined as at least 2 SIRS criteria plus 1 major Duke criterion (i.e. bacteraemia with a typical microorganism or evidence of endocardial involvement by echocardiography), presenting in the emergency room. Blood was simultaneously drawn for BC (Bact/ALERT FA/FN, bioMérieux) and for SeptiFast (1.5 ml EDTA-blood). If the heart valve was removed, culture, histology and broad-range PCR were performed. Patients were retrospectively classified according to Duke criteria by 2 independent investigators, blinded to the SeptiFast results, into confirmed or rejected IE.

**Results:** In this ongoing study (07–12/2008), 23 patients were included, of whom IE was confirmed in 9 (39%) and rejected in 14 (61%). Among 9 patients with confirmed IE (median age 50y, range 34–80y, 44% males), BC grew the pathogen in 6 (67%) including *Staphylococcus aureus* (n=3), *Streptococcus mitis* (n=2) and *S. gallolyticus* (n=1); SeptiFast detected pathogens in 8 of 9 IE-patients (89%), among whom 6 matched BC, 1 patient was positive in SeptiFast only (*Escherichia coli*) and 1 in broad-range PCR of the valve (*S. agalactiae*). In 1 patient with negative BC and negative SeptiFast, *Haemophilus* sp. was detected by broad-range PCR of the valve. 2 of 3 patients with negative BC received antibiotics before blood collection, among whom both were positive by SeptiFast. IE involved 4 native, 3 prosthetic (all aortic) and 1 native – prosthetic valve; in 1 patient the valve was not involved, but 3 minor Duke criteria were fulfilled.

**Conclusions:** BC detected 6 of 9 (67%) organisms causing IE, whereas SeptiFast 8 of 9 (89%). SeptiFast detected all 8 organisms for which specific primers are included in the test kit and deserves further investigation in the diagnosis of intravascular infections. A modified primer set may further improve detection of organisms causing IE, including culture-negative IE.

**P1833 Multiplex PCR (SeptiFast℠) for diagnosis of sepsis in patients presenting to the emergency room**


**Background:** Blood cultures (BC) identify the aetiology of sepsis in only a minority of patients and results are usually available ≥1 days later. More rapid and sensitive tests are needed. We assessed the utility of the multiplex real-time PCR SeptiFast℠ (SF) (Roche Diagnostics) to detect microbial DNA of the 25 pathogens of sepsis within 6h.

**Methods:** In this prospective ongoing study, we included unselected adult patients presenting to the emergency room with suspected sepsis, defined as core temperature >38.3°C or <36.0°C and ≥1 additional SIRS criterion. BC (Bact/ALERT, FA/FN, bioMérieux) and SF were simultaneously drawn at presentation and 0-1-2-4-8 h thereafter. The final diagnosis of sepsis or non-infectious SIRS was adjudicated by 2 independent investigators unaware of the SF result.

**Results:** To date (06/07–12/08), 88 patients with suspected sepsis were included (median age, 65 years; range 29–96 years; 48% males), of whom 74 (84%) had a confirmed sepsis and 14 (16%) were diagnosed as non-infectious SIRS. The severity of sepsis was classified as sepsis without organ dysfunction in 59 (80%), severe sepsis in 11 (15%) and septic shock in 4 (5%) patients. The infectious foci were pulmonary (34%), urinary tract (23%), abdominal (12%), skin (9%), ENT (7%), musculoskeletal (5%), other (4%) and not identifiable (8%). The causative organism was identified in 40 (54%) sepsis patients by conventional microbiology, including 21 (28%) with positive BC (14 *E. coli*, 3 *K. pneumoniae*, 3 *S. aureus*, 1 *S. pyogenes*), Median time to positivity was 17 h (range 6–45h). SF revealed the causative organism in 17 (23%) patients with sepsis, of which 14 matched the BC result.
3 samples were positive in SF only (E. coli – cholangitis, E. coli – urosepsis, S. aureus – primary sepsis), whereas 7 samples were positive in BC only (5 E. coli – urosepsis, 1 S. aureus – implant infection, 1 S. pyogenes – erysipelas). In patients with non-infectious SIRS, BC were positive in 2 patients (both contamination with coagulase-negative staphylococci), while SF remained negative. Table shows the overall test performance (%) of BC and SF in diagnosis of sepsis.

Conclusions: In patients with confirmed sepsis, BC detected the causative organism in 21 (28%) and SF in 17 (23%). BC missed the causative organism in 3 and SF in 7 sepsis patients, reflecting discontinuous bacteraemia/DNAemia in these patients or limited sensitivity of the respective assay. SF warrants further evaluation for the diagnosis of sepsis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Blood culture (BC)</th>
<th>SeptiFast® (SF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>28%</td>
<td>23%</td>
</tr>
<tr>
<td>Specificity</td>
<td>86%</td>
<td>100%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>91%</td>
<td>100%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>19%</td>
<td>20%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>38%</td>
<td>35%</td>
</tr>
</tbody>
</table>

**Methods:** We conducted a retrospective study to compare the results of conventional microbiology with real-time PCR approach directly from explanted heart valve tissues infective endocarditis (IE).

**Objective:** Better, more efficient clinical management would flow from quicker identification of organisms in blood culture to taxon and/or species level. The Mobidiag Prove-it Sepsis PCR and microarray platform is designed to speciate the most common blood culture-related organisms within 3 hours of positive blood culture flagging by conventional systems.

**Methods:** We compared this system’s diagnostic performance with conventional identification systems in two major teaching hospitals in Helsinki and London.

**Results:** 3298 blood cultures were analysed, of which 2087 yielded a pathogen by conventional techniques. Of these, 320 organisms were not covered by appropriate probes, and 135 contained more than one organism. Prove-it Sepsis sensitivity and specificity were 98% and 96%, respectively, for blood cultures containing a single organism within the detection panel. The system provided a result on average 18 hours earlier than conventional systems. Of particular significance was its faultless ability to differentiate MRSA from MSSA and from coagulase negative staphylococci. Investigation of discrepant results revealed 30 cases where the system’s sensitivity limits were likely exceeded; other discrepant cases related to human error, likely contamination during the extraction stage, and the system’s limitations relating to blood cultures containing more than one organism. Other issues relating to batch reagent variation were also identified and corrected within the trial timeframe. The system proved to be fast, reliable, robust, and rapid, with biochip analysis taking less than 10 seconds per sample.

**Conclusion:** Both centres identified cases where timely information which only this system could provide would have significantly improved patient management. Examples here include more rational antibiotic choice both through rapid differentiation of MRSA from either S. aureus or coagulase negative staphylococci, and speciation of Gram negative organisms. Once primers and probes for additional targets (specifically Candida spp.) are validated, we aim to perform a cost/benefit trial where decisions made with results provided by this simple, rapid, and robust diagnostic platform will be analysed for their impact on better patient outcome.

**Method:** Comparison of molecular biology-based methods and culture methods in diagnosing infectious endophthalmitis

**Objective:** Rapid detection of the infectious pathogen is crucial when confronted with a patient with endophthalmitis. Conventional culture methods used to be the standard. Developments in molecular biology allow new approaches in the diagnostic of ocular fluids such as PCR of intraocular fluids. Especially broad-range PCR testing for 16S ribosomal genes have increased detection rates and help to decrease contamination. 18 months ago we changed our approach to endophthalmitis diagnostics and want to report first results.

**Methods:** Intraocular fluids (anterior chamber and/or vitreous) of patients presenting with acute or chronic endophthalmitis were obtained. Culture medium was inoculated with the undiluted specimen directly in the operating room. Remaining specimen was left in the sterile syringe and used for subbacterial PCR. A positive result was sequenced with specific primers.

**Results:** In 12 of 24 patients (50%) a specific pathogen could be detected with PCR within 1−2 days. Later the results could be confirmed by culture. There were no discrepancies between both methods regarding the detected pathogen. In 12 patients no pathogen could be identified with either method.
Conclusions: Compared to culture PCR allows a faster detection of microbes in endophthalmitis patients. This is very important regarding the therapeutical consequences. The number of cases with negative results in both methods remains high in our study.

Methods: Analysis was performed using standard PCR, electrophoresis using E-Gel, Sanger sequencing on a ABI 3730 DNA analyzer, and principal component analysis (PCA) for motif pattern analysis.

Results: Both pharyngeal and invasive isolates were mainly emm-3 and prophage profile, phi-G3.01. In 1993 scIB harbouring 5-CAAAA tandem repeats emerged and by 2003, this was the only number of in-frame repeats observed. The variable collagen structural motif (CSM) region of ScIB was highly modular mainly consisting of two novel motifs; MC1CSMR-1 and -2, organised into unique motif-patterns for most of the strains. A third motif MC1CSMR-3 was also found for eleven strains, none of which originated from the same geographical location. It was not possible to discern between motif patterns produced by invasive or pharyngeal strains by PCA.

Conclusion: Taken together, the Norwegian emm-3 GAS are relatively homogenous, dominated by the widespread subtype emm-3-1, and the prophage profile phi-G3.01. It also became clear that a successful strain variant harbouring a scIB with 5 CAAAA repeats emerged in 1993 and was associated with the increase in emm-3 cases of invasive disease. Furthermore, the CSM region of ScIB was found to be consisting of two novel emm-3 specific conserved motif repeats, displaying highly variable motif patterns.
Inactivation of the transcriptional regulator mutR gene affects virulent phenotype of Streptococcus pyogenes

A.A. Zutkis*, A.V. Dmitriev, M.S. Chaussée, A.A. Totolian (St. Petersburg, RU; Vermillion, US)

Objectives: Streptococcus pyogenes (group A streptococcus) is Gram-positive pathogen exclusively adapted to humans. It is able to colonise numerous organs and tissues and cause a variety of diseases. In order to adapt changing environmental conditions, S. pyogenes employs different mechanisms of gene regulation, in particular, by the global genome transcriptional regulators, such as Mga, Rgg, RofA-like proteins and others. The MutR protein, which is encoded by the mutR gene is a putative transcriptional regulator and belongs to Rgg-family of regulators. The goal of this study is to analyze the potential role of MutR protein in S. pyogenes virulence.

Methods: A total of 20 S. pyogenes strains of different serotypes were used. The methods of molecular microbiology and genetic engineering were employed for cloning of the mutR gene fragment. Inactivation of the mutR gene in strain SF370 was done by insertional mutagenesis. Growth of the strains was analyzed in Todd-Hewitt broth. Virulent properties of the mutR mutant and parental SF370 strain were analyzed using intraperitoneal model of streptococcal infection in laboratory mice. Virulence was assessed by the lethal coefficient.

Results: The mutR gene was revealed in all the strains under analysis. The SacI-PstI internal fragment of the mutR gene was cloned in the vector, which is unable to replicate in Gram-positive cocci. This recombinant plasmid was used to inactivate the mutR gene in strain SF370. Erythromycin resistant clones were screened and the insertion of recombinant plasmid in the chromosomal DNA was confirmed by PCR and nucleotide sequencing. Growth curves of the mutR isogenic mutant and parental SF370 strain were analyzed in Todd-Hewitt broth. The strains demonstrated significant difference in the growth rate; the lag-phase of the mutant strain was 30 minutes, compared to 60 minutes for the wild-type strain. The maximum OD600 values reached were 1.1 and 0.8 for the mutR mutant and wild-type strain, respectively. Two infectious doses, 1×10^8 and 5×10^8 CFU/animal, were used for intraperitoneal model of streptococcal infection. With these infectious doses, the wild-type strain demonstrated the lethal coefficients equal to 0.13 and 0.22, respectively. The virulence of mutR isogenic mutant was dramatically decreased: neither 1×10^6 nor 5×10^5 CFU/animal resulted in the death of laboratory mice.

Conclusion: The MutR transcriptional regulator plays an important role in the virulence of S. pyogenes.

Comparison of Gene Xpert real-time PCR and various culture methods for the detection of group B Streptococcus in rectovaginal samples from antenatal women

A. Curry*, A. Walsh, M. Matheson, E. Collins, A. Donegan, S. Knowles (Dublin, IE)

Group B Streptococci (GBS) is an important cause of maternal and neonatal infection. The GeneXpert real time polymerase chain reaction (PCR) was evaluated for the detection of GBS and was compared with various culture methods.

Four ATCC strains: GBS, Enterococcus faecalis, E. coli and Staphylococcus aureus in pure and mixed cultures were initially trialled on the GeneXpert. Low vaginal/rectal GBS screening of 102 antenatal women, using a double-swab, were compared using the GeneXpert and 5 different culture methods. One swab was inoculated directly onto blood, ChromID CPS, neomycin blood and Granada agars and then placed in Todd Hewitt enrichment broth. The enrichment broth was subcultured onto ChromID CPS following overnight incubation. All suspect colonies underwent streptococcal grouping in order to confirm the presence or absence of GBS. The second swab was used for the Gene Xpert PCR.

All ATCC strains gave the expected results for the presence or absence of GBS in both pure and mixed cultures using the PCR system. Among patient samples, culture was used as the ‘Gold Standard’. The Gene Xpert PCR and ChromID CPS agar following enrichment in Todd Hewitt showed good sensitivity at 92% and 96% respectively. Direct culture methods on the various agar showed lower sensitivity for the detection of GBS, with sensitivity of direct culture on ChromID CPS lowest at 61% and highest on Granada agar at 83%. In two women the Gene Xpert PCR was positive and the culture negative for GBS. The specificity of ChromID CPS following enrichment in Todd Hewitt was 100%; Granada agar 99%; Gene Xpert PCR 98% and direct culture of ChromID CPS 97%. All methods gave good negative predictive values ranging from 97–99%. Direct culture of ChromID CPS agar gave the lowest positive predictive value at 82% but this increased to 100% following enrichment; the Gene Xpert PCR was 92% and Granada agar 95%.

The Gene Xpert is a rapid method taking approximately 1.5 hours from receipt of specimen to result, compared with culture methods, which can take 24–48 hours. However it is more expensive and culture must be retained in order to perform susceptibility testing if the patient is penicillin allergic. In conclusion, the Gene Xpert is a sensitive and specific method which provides rapid results for the presence of GBS in antenatal women and may be useful for rapid intrapartum testing.
Inactivation of DNA-binding response regulator Sak189

Characterisation of group G streptococcal strains recovered from humans

Method: S. agalactiae strain 168-00 was used in the study. sak188 and sak189 isogenic mutants were constructed by insertional mutagenesis. Microbiological and biochemical properties of the mutant strains were compared with those of 168-00. Cell lysates were analysed by SDS-PAGE and western blotting. Virulence properties of the strains were assessed using intraperitoneal model of streptococcal infection in laboratory mice. Results: DNA fragments of sak188 and sak189 genes were cloned in the vector unable to replicate in streptococci. These recombinant plasmids were used to construct sak188 and sak189 gene in the strain 168-00, respectively. Analysis of the growth in Todd-Hewitt broth revealed slightly slower growth of the mutant strains compared to the parental strain. SDS-PAGE demonstrated no difference among the mutant strains and wild-type strain, with the exception of a single band of about 140 kDa, which was present in wild-type strain and sak188 mutant, but not in sak189 mutant. Western blotting identified this protein as β-antigen. Experimental streptococcal infection in vivo demonstrated an increase in virulence properties of sak189 mutant compared to the wild-type strain and sak188 mutant. Conclusion: DNA-binding response regulator Sak189 is necessary for β-antigen expression. Inactivation of the sak189 gene affects S. agalactiae virulence.

Characterisation of group G streptococcal strains recovered from humans

Background: Group G streptococci form a heterogeneous group of microorganisms. In humans, they may colonise pharynx, skin, gastrointestinal and female genital tract. However, they have been reported with increasing frequency as a cause of variety of human infections with clinical manifestations similar to infections caused by S. pyogenes. Recently, group G streptococci have been associated with severe invasive infections such as necrotising fasciitis and toxic shock syndrome.

Method: In present study 200 strains of group G streptococci isolated from ambulant and hospitalised patients in Pardubice were collected. Strains were identified using PCR aimed at species-specific parts of the 16S-23S rDNA intergenic spacer region. Isolated strains were further searched for genes encoding streptococcal exotoxins including those with superantigenic or mitogenic activities (sagA, speG, smaZ). Antimicrobial susceptibilities were determined by microdilution method according to the CLSI recommendations.

Results: Out of 200 group G streptococcal strains, 185 were identified as S. dysgalactiae ssp. equisimilis and 5 as S. canis. The saga gene was detected in 97% S. dysgalactiae ssp. equisimilis and 60% of S. canis strains. All group G streptococcal strains were PCR-negative for the presence of speG and smaZ using primers that anneal to structural gene sequences. All strains were susceptible to penicillin (MIC ≤ 0.063 μg/ml), ampicillin (MIC ≤ 0.125 μg/ml), ampicillin/sulbactam (MIC ≤ 0.125 μg/ml), ceftazidin (MIC ≤ 0.125 μg/ml), nitrofurantoin (MIC ≤ 16 mg/l), tetracyclin (MIC ≤ 0.5 mg/l) and vancomycin (MIC ≤ 0.5 mg/l). The least effective antimicrobial agent was found to be tetracycline (MIC > 16 mg/l).

Conclusion: Although group G β-haemolytic streptococci don’t belong among common streptococcal species, their importance should not be underestimated. S. dysgalactiae ssp. equisimilis is associated with 5–8% of human streptococcal infections, including serious, life-threatening states. S. canis, important animal pathogen, may cause similar symptoms when infecting human. This work was supported by MSM 0021627502.

Telavancin activity against S. aureus and coagulase-negative staphylococci collected from clinical infections in Europe (2007–2008)

H. Sader, P. Rhomberg*, R. Jones (North Liberty, US)

Objectives: To assess the activity of telavancin, a potent investigational lipoglycopeptide agent with a multifunctional mechanism of action, and rapid bacteraidal activity, against Gram-positive bacterial pathogens. We compared the antimicrobial potency of telavancin to key comparator agents by testing contemporary European (EU) clinical strains of S. aureus and coagulase-negative staphylococci (CoNS).

Methods: A total of 7,534 non-duplicate staphylococci including 5,726 S. aureus (oxacillin-resistant [OX-R], 27.5%) and 1808 CoNS (OX-R, 76.8%) were submitted in 2007 and 2008 as part of a Telavancin Surveillance Program from 28 medical centres in 11 EU countries, Turkey (2 sites) and Israel (1 site). Susceptibility (S) testing was performed on all strains using CLSI broth microdilution methods (CLSI, M7-A7 2006).
Results: All EU staphylococcal strains were inhibited by telavancin at ≤0.5 mg/L. For both S. aureus (MIC50/90, 0.12/0.25 mg/L) and CoNS (MIC50/90, 0.12/0.25 mg/L), telavancin MIC results were identical when comparing OX-S and OX-R subsets. There was no variation in MIC results for telavancin among S. aureus strains based on source of infection or by the eight major CoNS species groups analyzed. Highest vancomycin MIC values for CoNS species were observed for S. haemolyticus strains. Highest teicoplanin MIC values among CoNS were among S. haemolyticus and S. capitis strains. S. aureus strains from Ireland/United Kingdom had the highest MRSA rate (39.5%) followed by Italy (28.9%), France (26.5%), Spain (23.8%) and Germany (16.2%). CoNS teicoplanin-R rates ranged from 1.7% (Italy), 0.7% (France), and 0.3% (Ireland/United Kingdom) to 0.0% (Germany and Spain). Telavancin activity against staphylococci is summarised in the Table.

Conclusions: Telavancin was highly active (all MIC values at ≤0.5 mg/L) against both S. aureus and CoNS isolates collected in EU medical centres during 2007 and 2008. No variation in telavancin MIC was observed for staphylococci when categorised by oxacillin susceptibility, infection site, species or geographic origin. Continued surveillance to detect emergence of resistance is warranted to monitor this new lipoglycopeptide.

ORGANISM (NO. TESTED) MIC (MG/L) CUMULATIVE % INHIBITED AT MIC (MG/L) OF:

<table>
<thead>
<tr>
<th>Organism (no. tested)</th>
<th>No. of strains (cumulative %) inhibited at telavancin MIC (mg/L) of:</th>
<th>≤0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (3726)</td>
<td>10 (0.3)</td>
<td>382 (7.0)</td>
<td>372 (7.6)</td>
<td>1574 (99.4)</td>
<td>52 (100.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>OX-S (4150)</td>
<td>14 (0.3)</td>
<td>251 (6.4)</td>
<td>2765 (73.0)</td>
<td>1009 (99.5)</td>
<td>21 (100.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>OX-R (1376)</td>
<td>2 (0.1)</td>
<td>131 (4.8)</td>
<td>977 (65.2)</td>
<td>475 (95.5)</td>
<td>11 (100.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>CoNS (1388)</td>
<td>12 (0.7)</td>
<td>15 (11.1)</td>
<td>1600 (66.6)</td>
<td>566 (97.9)</td>
<td>38 (100.0)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>OX-S (280)</td>
<td>2 (0.5)</td>
<td>9 (2.6)</td>
<td>56 (18.0)</td>
<td>236 (72.1)</td>
<td>113 (99.1)</td>
<td>4 (100.0)</td>
<td></td>
</tr>
<tr>
<td>OX-R (3188)</td>
<td>10 (0.7)</td>
<td>4 (1.3)</td>
<td>120 (37.1)</td>
<td>767 (47.6)</td>
<td>34 (100.0)</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Table: Antimicrobial activity of telavancin tested against streptococcal species isolated from European medical centres (2007-2008)

P. Rhomberg*, H. Sader, R. Jones (North Liberty, US)

Objectives: To determine the antimicrobial potency and spectrum of activity of telavancin and key comparator agents against Gram-positive organisms (Enterococcus spp. and rare species) isolated in European (EU) medical centres in 2007–2008. Telavancin is under FDA/regulatory review for skin and skin structure infections (SSSI) and hospital-acquired pneumonia (HAP) indication.

Methods: A total of 13,565 EU isolates of Gram-positive cocci were tested, of which 2,210 were enterococci (1,337 E. faecalis [EF]; 809 E. faecium [EFM]) and four other species having samples of >10 isolates (B. cereus [BC; 11], L. monocytogenes [LM; 14], M. luteus [ML; 17], Corynebacterium spp. [CS; 30]). All 2,282 isolates were processed by reference broth microdilution method in a central laboratory (JMI Laboratories) with validated identifications and concurrent QC procedures per CLSI M100-S18 (2008).

Results: Activity of telavancin versus the most prevalent enterococci are listed in the Table.

Table: Antimicrobial activity of telavancin tested against enterococci and uncommonly isolated Gram-positive species (European sample for 2007–2008)

R. Jones, P. Rhomberg*, H. Sader (North Liberty, US)

Objectives: To determine the potency and spectrum of activity of telavancin, an investigational lipoglycopeptide, against selected Gram-positive organisms (Enterococcus spp. and rare species) isolated in European (EU) medical centres in 2007–2008. Telavancin is under FDA/regulatory review for skin and skin structure infections (SSSI) and hospital-acquired pneumonia (HAP) indication.

Methods: A total of 13,565 EU isolates of Gram-positive cocci were tested, of which 2,210 were enterococci (1,337 E. faecalis [EF]; 809 E. faecium [EFM]) and four other species having samples of >10 isolates (B. cereus [BC; 11], L. monocytogenes [LM; 14], M. luteus [ML; 17], Corynebacterium spp. [CS; 30]). All 2,282 isolates were processed by reference broth microdilution method in a central laboratory (JMI Laboratories) with validated identifications and concurrent QC procedures per CLSI M100-S18 (2008).

Results: Activity of telavancin versus the most prevalent enterococci are listed in the Table.

Table: Antimicrobial activity of telavancin tested against enterococci and uncommonly isolated Gram-positive species (European sample for 2007–2008)

P. Rhomberg*, H. Sader, R. Jones (North Liberty, US)

Objectives: To determine the antimicrobial potency and spectrum of activity of telavancin and key comparator agents against Gram-positive organisms (Enterococcus spp. and rare species) isolated in European (EU) medical centres in 2007–2008. Telavancin is under FDA/regulatory review for skin and skin structure infections (SSSI) and hospital-acquired pneumonia (HAP) indication.

Methods: A total of 13,565 EU isolates of Gram-positive cocci were tested, of which 2,210 were enterococci (1,337 E. faecalis [EF]; 809 E. faecium [EFM]) and four other species having samples of >10 isolates (B. cereus [BC; 11], L. monocytogenes [LM; 14], M. luteus [ML; 17], Corynebacterium spp. [CS; 30]). All 2,282 isolates were processed by reference broth microdilution method in a central laboratory (JMI Laboratories) with validated identifications and concurrent QC procedures per CLSI M100-S18 (2008).

Results: Activity of telavancin versus the most prevalent enterococci are listed in the Table.

Table: Antimicrobial activity of telavancin tested against enterococci and uncommonly isolated Gram-positive species (European sample for 2007–2008)

P. Rhomberg*, H. Sader, R. Jones (North Liberty, US)

Objectives: To determine the antimicrobial potency and spectrum of activity of telavancin and key comparator agents against Gram-positive organisms (Enterococcus spp. and rare species) isolated in European (EU) medical centres in 2007–2008. Telavancin is under FDA/regulatory review for skin and skin structure infections (SSSI) and hospital-acquired pneumonia (HAP) indication.

Methods: A total of 13,565 EU isolates of Gram-positive cocci were tested, of which 2,210 were enterococci (1,337 E. faecalis [EF]; 809 E. faecium [EFM]) and four other species having samples of >10 isolates (B. cereus [BC; 11], L. monocytogenes [LM; 14], M. luteus [ML; 17], Corynebacterium spp. [CS; 30]). All 2,282 isolates were processed by reference broth microdilution method in a central laboratory (JMI Laboratories) with validated identifications and concurrent QC procedures per CLSI M100-S18 (2008).

Results: Activity of telavancin versus the most prevalent enterococci are listed in the Table.

Table: Antimicrobial activity of telavancin tested against enterococci and uncommonly isolated Gram-positive species (European sample for 2007–2008)

P. Rhomberg*, H. Sader, R. Jones (North Liberty, US)

Objectives: To determine the antimicrobial potency and spectrum of activity of telavancin and key comparator agents against Gram-positive organisms (Enterococcus spp. and rare species) isolated in European (EU) medical centres in 2007–2008. Telavancin is under FDA/regulatory review for skin and skin structure infections (SSSI) and hospital-acquired pneumonia (HAP) indication.

Methods: A total of 13,565 EU isolates of Gram-positive cocci were tested, of which 2,210 were enterococci (1,337 E. faecalis [EF]; 809 E. faecium [EFM]) and four other species having samples of >10 isolates (B. cereus [BC; 11], L. monocytogenes [LM; 14], M. luteus [ML; 17], Corynebacterium spp. [CS; 30]). All 2,282 isolates were processed by reference broth microdilution method in a central laboratory (JMI Laboratories) with validated identifications and concurrent QC procedures per CLSI M100-S18 (2008).

Results: Activity of telavancin versus the most prevalent enterococci are listed in the Table.

Table: Antimicrobial activity of telavancin tested against enterococci and uncommonly isolated Gram-positive species (European sample for 2007–2008)
Efficacy of oritavancin at single or infrequent doses for the treatment of complicated skin and skin-structure infections


Objective: Oritavancin (Ori) is a semisynthetic lipoglycopeptide with demonstrated efficacy against Gram-positive complicated skin and skin structure infections (cSSSI) including methicillin-resistant Staphylococcus aureus (MRSA), when given 200 mg IV daily for 3–7 days or in a daily-dose fashion. Animal and phase 2 and 3 pharmacokinetic data suggest potential for single dose or infrequent dose. Efficacy of single and infrequent dose was similar to predetermined times. Clinical efficacy was assessed at end of therapy, test of cure (TOC), & late follow-up (LFS).

Results: 302 patients received Ori (100 daily dose; 99 single dose; 103 infrequent dose). Efficacy of single and infrequent dose was similar to daily dose at TOC. The rate of treatment emergent adverse events was similar between dose groups and all were well tolerated.

**Conclusion:** Single and infrequent doses of oritavancin were as efficacious as daily doses for cSSSI caused by Gram-positive pathogens, including MRSA. Safety and tolerability were similar among dosing groups.

Oritavancin in the treatment of immunocompromised patients with complicated skin and skin-structure infections


Objective: Oritavancin is a novel semisynthetic lipoglycopeptide with multiple mechanisms of action and rapid, bactericidal activity against a wide range of Gram-positive bacteria, including those resistant to currently existing therapies, and is presently under regulatory review for the treatment of complicated skin and skin structure infections (cSSSI). Patients with diabetes mellitus (DM) have increased risk and prevalence of SSSI and frequently have co-morbidities contributing to increased morbidity with lower cure rates. We compared the efficacy and safety of oritavancin to vancomycin for cSSSI in patients with DM.

Methods: Two randomised, double-blind, multicentre, phase 3 trials were designed to test whether 3 to 7 days of oritavancin (ORI) was noninferior to 10 to 14 days of vancomycin/cephalexin (VAN) in the treatment of patients with cSSSI. Patients randomised to ORI received 3–7 days of IV drug then 7–11 days of IV/oral placebo. Patients randomised to VAN received 3–14 days of IV drug then 0–11 days of oral cephalaxin. ORI was dosed at 1.5 mg/kg or 3.0 mg/kg in 1 study and 200 mg/d (300 mg for patients >110 kg) in the other. VAN was dosed at 15 mg/kg q 12h, dose-adjusted based on creatinine clearance. Test-of-cure (TOC) occurred from Days 21 to 35. Described here are results from the subset of patients with DM.

**Results:** 1763 patients received study medication, of which 386 had DM. In clinically evaluable (CE) patients, mean duration of total active dosing was 5.2 days in the ORI group compared to 11.3 days in the VAN group. Clinical cure rates in the non-DM patients in the CE population at TOC were 80.9% (597/738) in ORI patients compared to 79.2% (286/361) for VAN patients (95%CI: –3.4, 6.7). Consistent with reports of lower response rates for infections in diabetics, clinical cure rates in DM patients in the CE population at TOC were 62.2% (123/201) in ORI patients compared to 62.9% (61/97) for VAN patients (95%CI: –12.4, 11.0). Overall, higher percentages of patients with DM had at least one adverse event (61.5% for ORI and 69.0% for VAN) than those who did not (51.3% for ORI and 60.6% for VAN), as is clinically expected. Laboratory changes were comparable between the ORI and VAN groups with and without DM.

**Conclusion:** Oritavancin is a novel lipoglycopeptide with demonstrated efficacy and a favourable safety profile with short course therapy (3–7 days) in the treatment of cSSSI in patients with diabetes.

Hepatic insufficiency and outcomes in patients with complicated skin and skin-structure infections treated with oritavancin


Objective: Oritavancin (ORI) is a novel semisynthetic lipoglycopeptide with activity against a wide range of Gram-positive bacteria, including those resistant to currently existing therapies. Pharmacokinetics of ORI in mild or moderate hepatically impaired subjects do not indicate a need for dose adjustment. Therefore, ORI outcomes are not expected to be affected by liver disease.
Methods: Two randomised, double-blind, multicentre, phase 3 trials were designed to test whether 3 to 7 days of oritavancin (ORI) was noninferior to 10 to 14 days of vancomycin/cephalexin (VAN) in the treatment of complicated skin and skin structure infections (cSSSI). Test-of-cure (TOC) occurred between Days 21 to 35. Outcomes were analyzed for patients with hepatic insufficiency at baseline. Patients were identified through the reporting of preexisting conditions or adverse events (AEs) prior to baseline by searching for preferred terms related to hepatic insufficiency.

Results: 1763 patients received study medication, 1173 ORI and 590 VAN. 38 ORI patients and 21 VAN patients had hepatic insufficiency. Clinical cure rates in non-hepatically impaired patients in the clinically evaluable (CE) population at TOC were 76.9% (698/908) in ORI compared to 76.3% (338/443) for VAN. Similar response rates were observed in hepatitis impaired patients with clinical cure rates in the CE population at TOC of 77.4% (24/31) in ORI compared to 60.0% (9/15) for VAN. Overall, more patients with hepatic insufficiency had at least one AE (71.1% for ORI and 76.2% for VAN) than patients without insufficiency (53.0% for ORI and 61.9% for VAN) as is clinically expected. Laboratory changes were similar between ORI and VAN treatment groups in patients with and without hepatic insufficiency.

Conclusions: Oritavancin is a novel lipoglycopeptide with demonstrated efficacy and a favourable safety profile with short course therapy (3–7 days) in the treatment of cSSSI that is not affected by liver disease.
Comparative activity of oritavancin against recent genetically diverse methicillin-resistant *Staphylococcus aureus* isolates

**Methods:** Strains (n = 58) were clinical isolates obtained between 2005 and 2006 from hospitals in the New York/New Jersey, USA area. Broth microdilution MICs were determined according to CLSI guidelines. Identification of the Panton-Valentine leukocidin (pvl) genes was by polymerase chain reaction (PCR). Multiplex PCR was used for staphylococcal cassette chromosome (SCC) mec typing. *Staphylococcus* protein A (spa) typing was by PCR followed by sequencing of the PCR products.

**Results:** The MRSA isolates were genetically diverse and included classical community-associated USA300 isolates (n = 17), PVL+ strains (n = 33) and PVL- strains (n = 25). Majority (95%) of the PVL+ isolates were typed as SCCmec IV. Of the PVL- isolates, 60% were SCCmec II and 40% were SCCmec IV. ORI MIC90s were identical for the isolates and sepsis again drug-resistant isolates of *S. pyogenes*.

### Objectives

Oritavancin (ORI) is a lipoglycopeptide with bactericidal activity against Gram-positive bacteria including drug-resistant *S. aureus*. This study describes ORI activity against recent genetically diverse MRSA isolates compared to vancomycin (VAN), teicoplanin (TEI), oxacillin (OXA), erythromycin (ERY), daptomycin (DAP), ciprofloxacin (CIP) and linezolid (LIN).

### Conclusions

ORI activity was unaffected by presence of PVL, SCCmec type or spa type. Based on MIC90s, ORI was more potent than the comparators used in this study. ORI and linezolid (LIN) demonstrated bactericidal by between 30 min and 3 h. When tested at its free trough concentration, ORI demonstrated bactericidal against recent *S. pyogenes* isolates and is effective against drug-resistant isolates of *S. pyogenes*.

**Epidemiology of MRSA**

**Objectives:** MRSA is an important cause of both nosocomial and community-acquired infections. Severe MRSA infections, including necrotising pneumonia, bacteremia and skin and soft tissue infections (SSTIs) have been associated with the virulence factor Panton-Valentine leukocidin (PVL). The aim of this study was to investigate the presence of PVL genes, clonality and their susceptibility patterns in MRSA isolates collected from patients in our institution from October 2007 to December 2008.

**Methods:** All MRSA strains isolated during this period of time were screened for PVL genes. Genotype characterisation of PVL was made by co amplification of the genes lukS-PV and lukF-PV by PCR. Biochemical and antimicrobial susceptibility of isolates were performed by Phoenix® (Becton Dickinson, Franklin Lakes, NJ, USA). Glycopeptides, daptomycin and linezolid MICs were determined by Etest® (AB Biodisk, Solna, Sweden). All MRSA PVL positive isolates were genotyped by pulsed field gel electrophoresis (PFGE) after digestion with SmaI.

**Results:** 213 MRSA isolates were collected, 24 (11.3%) were PVL positive. Strains were isolated from cutaneous abscesses (7), ulcer infection (5), cellulitis (5), folliculitis (4), surgical site infection (2) and nasal swab (1). Ten different susceptibility patterns were found. Resistance only to penicillin and oxacillin was observed in 5 isolates. All isolates were susceptible to cefotaxime, rifampin, vancomycin, teicoplanin, daptomycin, fusidic acid and linezolid. MIC90 for vancomycin, teicoplanin, daptomycin and linezolid were 2, 0.75 and 0.5 mcg/mL, respectively. Patients carrying PVL positive MRSA
strains (24) were from Spain (16), USA (2), France (1), Italy (1), Cuba (1), Brazil (1), Ecuador (1) and Argentina (1). Among these strains, nine \(^{19}\)PFGE patterns were observed.

**Conclusion:** MRSA PVL strains are an increasing problem due to its involvement in SSTIs. In our institution these isolates represent 11.3% of MRSA strains. Recent marketed anti-staphylococci antibiotics such as daptomycin and linezolid demonstrated good activity against these particular MRSA isolates.

### \(P1859\) Comparison of clinical features and mortality risk associated with pneumonia due to community-acquired methicillin-resistant and methicillin-susceptible \textit{Staphylococcus aureus} producing the Panton-Valentine leukocidin

**K. Vardakas*, D. Matthaiou, M. Falagas (Athens, GR)

**Objective:** Studies comparing patients with methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and methicillin-sensitive \textit{S. aureus} (MSSA) community-acquired pneumonia (CAP) are not forthcoming. The present review was undertaken to investigate the clinical features and prognosis of patients with MRSA CAP as compared to those of patients with MSSA CAP producing the Panton-Valentine leukocidin (PVL).

**Methods:** PubMed and Scopus were searched to identify articles that studied patients with MRSA CAP. Inclusion was stratified according to \textit{S. aureus} susceptibility, clinical, microbiological, and outcome data regarding individual patients or group of patients with \textit{S. aureus} CAP; both primary and secondary cases of CAP (haematogenous spread from other sites of infection) could be included.

**Results:** We identified 74 and 63 articles reporting data on MRSA and MSSA CAP, respectively. 107 patients had \textit{S. aureus} CAP due to PVL positive strains (76 MRSA and 31 MSSA). There were no differences in demographics and history among patients with MRSA and MSSA CAP. The features associated with MRSA CAP were gastrointestinal tract symptoms (p < 0.016) and unilobar infiltrates (p = 0.043). The features associated with MSSA CAP were airway haemorrhage (p = 0.010), multilobar infiltrates (p = 0.043) and acute respiratory distress syndrome (ARDS, p = 0.023). ARDS was the only independently associated variable with MSSA CAP in the multivariate analysis. Although MSSA patients were more likely to receive initial appropriate antimicrobial therapy (p < 0.001), there was no difference in mortality between the two groups (p = 0.919). Univariate analysis showed that respiratory disease (p = 0.027), influenza like symptoms (p < 0.001), rash (p = 0.024), septic shock (p = 0.010), mechanical ventilation (p < 0.001), multi-organ failure (p < 0.001), pleural effusion (p = 0.034), ARDS (p = 0.021), lung abscess (p = 0.025), leucopenia (p < 0.001), use of macrolides after microbiological cultures (p = 0.011), admission to the ICU (p < 0.001), and necrotising characteristics of CAP (p = 0.026) were the factors associated with mortality.

**Conclusions:** Patients with MRSA CAP did not have higher mortality than did patients with MSSA CAP.

### \(P1860\) Do laboratory characteristics predict outcome in methicillin-resistant \textit{Staphylococcus aureus} bacteraemia?

**S.M. Donabedian*, C.L. Moore, M.B. Perri, T. Chua, M.J. Zervos (Detroit, US)

**Objectives:** To determine if laboratory characteristics, such as susceptibility testing and molecular analysis, are associated with outcome in methicillin-resistant \textit{Staphylococcus aureus} bacteraemia (MRSA-B).

**Methods:** We identified consecutive subjects with MRSA-B empirically treated with vancomycin (VAN) and lab characteristics of the index strains were evaluated. Susceptibility testing for VAN and daptomycin (DAP) and pulsed-field gel electrophoresis (PFGE) for strain types was conducted. Heteroreistance (GISA/hGISA) was assessed using the macrodilution Etest. The objective was to correlate lab characteristics to a composite outcome of failure, defined by mortality 30 days from index culture, microbiologic failure (≥10 days of bacteremia), and/or recurrence of MRSA-B within 30 days of end of therapy. Predictors of failure were determined on univariate analysis and independent predictors were determined using stepwise logistic regression analysis.

**Results:** Subjects with MRSA-B (n = 189) were identified over a two year period. VAN MIC by broth microdilution (n = 185) was 0.25 (16.8%), 0.5 (69.2%), 1.0 (13.3%), 2.0 (0.5%). VAN MIC by Etest (n = 189) was 1.0 (5.8%), 1.5 (75.7%), ≥2 (18.5%). VAN MIC by Vitek (n = 189) was ≤1 (90.5%), 2 (9.5%). DAP MIC by Etest (n = 187) was 0.25 (2.7%), 0.5 (56.7%), 1 (39.0%), 2 (1.6%). PFGE (n = 189) were USA100 (49.2%), USA300 (40.2%), USA600 (2.5%) and other (7.9%). GISA/hGISA (n = 151) was found in 53%.

Failure occurred in 22.6% of subjects. Lab characteristics in the success group were compared to the failure group, respectively. VAN MIC by broth microdilution was 0.25 (19.4% vs 7.3%), 0.5 (69.4% vs 68.3%), 1.0 (11.1% vs 22%), 2.0 (0 vs 2.4%), p = 0.028. VAN MIC by Vitek was ≤1 (93.2% vs 81%) and 2 (6.8% vs 19%), p = 0.017. VAN and DAP MIC by Etest were not associated with outcome. PFGE was similar between groups except for USA600, where all 5 subjects failed. GISA/hGISA was more common in the failure success group (15.6% vs 2.5%), p = 0.003. GISA/hGISA was the only independent predictor for failure (OR 6.6; 95% CI 1.1–39.9, p = 0.04). VAN MIC by broth microdilution trended towards an independent association with failure (OR 5.2, 95% CI 0.9–30.3, p = 0.06).

**Conclusion:** USA600 strain type and higher VAN MIC, still within the susceptible range, by both broth microdilution and Vitek were associated with poor outcome. VAN and DAP MIC by Etest were not associated with outcome. GISA/hGISA was independently associated with failure.

### \(P1861\) A unique way to predict vancomycin failure in patients with methicillin-resistant \textit{Staphylococcus aureus} bacteraemia early in therapy: a classification and regression tree analysis

**C. Moore*, F. Cheema, T. Chua, P. Osaki Kyian, M. Perri, S. Davis, S. Donabedian, N. Haque, M. Zervos (Detroit, US)

**Objectives:** To determine the effect of a combination of lab, patient, and treatment (tx) factors on outcome of MRSA bacteraemia (MRSA-B) treated with vancomycin (VAN).

**Methods:** We conducted medical records review and lab analysis of consecutive subjects/strains with MRSA-B treated with VAN. Failure was a composite of: 30-day mortality, microbiologic failure (positive cultures ≥10 days from index culture) and/or recurrence of MRSA-B within 30 days of end of tx. Logistic regression (LR) was conducted. Classification and Regression Tree (CART) analysis was performed with a combination of baseline data and clinical/tx data on day 3 of VAN tx.

**Results:** Subjects with MRSA-B (n = 190) had a median age of 55y with 57.4% male. Risk level for source of infection was low (28.9%), intermediate (45.8%) and high (25.3%). Strains were USA100 (49.2%), USA300 (40.2%) and other (10.6%). VAN MIC by broth microdilution was 0.25 (16.8%), 0.5 (69.2%), 1.0 (13.5%), 2.0 (0.5%). VAN MIC by Etest (n = 189) was 1.0 (5.8%), 1.5 (75.7%), ≥2 (18.5%). Failure occurred in 22.6% of subjects. LR revealed high risk source (OR 3.1, 95% CI 1.6–6.0), peptic ulcer disease (OR 12.7, 95% CI 2.1–75.3), cardiovascular disease (OR 3.8, 95% CI 1.3–11.6) were independently associated with failure. Empiric synergy (OR 0.3, 95% CI 0.1–0.8) was associated with a positive outcome. Trends were seen in VAN MIC by broth (OR 5.3, 95% CI 0.9–30.6), immunosuppression (OR 3.3, 95% CI 1.0–10.8) and ICU at onset (OR 2.5, 95% CI 0.9–7.0).

CART analysis revealed in diagnosis subjects, the strongest predictor of failure was age with a cutoff of 73.5 years found by the analysis. In subjects with age >73.5 failure rate was 54.5%, whereas in subjects with age ≤73.5 failure rate was 6.9%. In non-diagnosis subjects, the strongest predictor for failure was initial VAN trough, with a cutoff of 22 ug/ml found. In subjects with trough ≥22, failure rate was 80%. In subjects with trough ≤22, the next best predictor of failure was creatinine clearance (CrCl). In subjects with CrCl ≥32, failure rate was 13.3%. In subjects with CrCl ≤32, failure rate was 55%.

**Conclusion:** Independent predictors of failure in MRSA-B are high risk source, peptic ulcer disease, cardiovascular disease, and malignancy.
Empiric synergy conveyed benefit in outcome. The CART analysis provides a useful clinical decision tree model to classify the likelihood of failure in subjects with MRSA-B at day 3 of VAN therapy.

**How does persistent bacteraemia affect outcome in methicillin-resistant *Staphylococcus aureus* bacteraemia?**

C. Moore*, T. Chua, F. Cheema, P. Osaki Kiyan, M. Perri, S. Donabedian, N. Haque, M. Zervos (Detroit, US)

**Objectives:** The impact of persistent bacteraemia (P-B, >7 days) on outcome of methicillin-resistant *Staphylococcus aureus* bacteraemia (MRSA-B) remains controversial. The primary objective of this study was to determine the effect of P-B on 30 day mortality. Secondly, we determined what association patient-specific, treatment, and lab data have on P-B.

**Methods:** We conducted medical records review and lab analysis of consecutive subjects with MRSA-B treated with vancomycin (VAN). Subjects were classified by duration of bacteraemia (>7d vs <7d). Stepwise logistic regression analysis was conducted to determine independent predictors of persistence.

**Results:** Subjects with MRSA-B (n 190) had a median age 55.0 and 57.4% male. Source of infection was low risk (28.9%), intermediate risk (45.8%) and high risk (25.3%). Median duration of bacteraemia was 4.0d (1−20). 30-day mortality was 13.2% and 7.7% had a recurrence of MRSA-B within 30 days of end of therapy. 20.5% had persistent bacteraemia. The group with bacteraemia <7 days (n 151) was compared to the P-B group (n 39). 30-day mortality was 10.6% vs 23.1%, p = 0.04. Factors significantly (p < 0.05) associated with P-B were baseline creatinine clearance <30 ml/min, left-sided endocarditis, initial vancomycin trough >15, higher VAN MIC by broth microdilution and USA600 strain type. Female sex and higher VAN AUC/MIC ratio (by broth microdilution) were associated with successful outcome (p < 0.05). Several factors demonstrated a trend (p < 0.1) towards association with P-B, including: older age, CHF, DM, higher VAN MIC by Etest and VAN MBC ≥32. Other factors were evaluated and showed no association with P-B, including: APACHE II score, risk level of source, multiple comorbidities, treatment characteristics such as synergy, heteroresistance and other PFGE types. Logistic regression analysis revealed left-sided endocarditis (OR 10.5; 95% CI 1.5–73.6, p = 0.02) and USA600 strain type (OR 18.5; 95% CI 1.2–274.8, p = 0.03) as independent predictors of P-B. Female sex (OR 0.18; 95% CI 0.05–0.6, p < 0.01) and every 100 unit increase in AUC/MIC (OR 0.83; 95% CI 0.7–0.96, p = 0.01) were associated with bacteraemia <7 d.

**Conclusion:** This study demonstrates that P-B is associated with higher 30-day mortality in subjects with MRSA-B. Left-sided endocarditis and USA600 strain type were independent predictors of P-B. Female sex and every 100 unit increase in AUC/MIC ratio were associated with clearance of bacteraemia within 7 days.

**Impact of mandatory surveillance on the measurement of methicillin-resistant *Staphylococcus aureus* bacteraemia in the English NHS: comparison of mandatory and voluntary surveillance reporting systems**

A. Pearson*, R. Guy, M. Murray (London, UK)

**Background:** The routine surveillance of bacteraemia in England is based on voluntary reporting of laboratory diagnosed cases to a national database (LabBase reporting system). In 2001 the Department of Health (DH) mandated an additional reporting system for methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia.

**Objective:** To report the impact of introducing a parallel mandatory reporting system for MRSA bacteraemia by comparison of reports made to the mandatory and voluntary systems.

**Methods:** The analysis reported here covers MRSA bacteraemia cases reported on the basis of specimens collected during the period 2002 to 2007. National data from the Healthcare Associated Infection data capture system (HCAl DCS) was extracted on the basis of the total annual reports received.

**Results:** The figure depicts the comparison of yearly MRSA bacteraemia reports. During the six year period 32,606 reports of MRSA bacteraemia were made to the voluntary national surveillance system (LabBase). The introduction of mandatory reporting to the web enabled HCAl DCS increased this number to 41,133. The impact of mandatory reporting had the greatest impact in the first year when reporting increased by 31.5% from 5529 to 7274 cases. Comparison of the reported numbers for the next four years showed between 25 and 27% differences between the mandatory and voluntary schemes. During the sixth year of mandatory surveillance the reporting difference dropped to 17% (718 cases).

**Conclusion:** Comparison of voluntary and mandatory reporting systems provides a tool for internal QC for mandatory surveillance. The marked fall in the differential of reported cases requires investigation to discern the extent to which this finding resulted from changes in ascertainment or was a result of the complex programme of target driven interventions.

Methods: All bacteraemia isolates of S. aureus from Denmark have been collected and stored since 1960. From this collection we chose 20 S. aureus strains, selected according to phage type (83A complex), clonal complex, time of isolation (a range from 1957 to 1980 represented) and with varying antibiotic resistance profiles. The resistance genotypes for macrolide- and tetracycline resistance were determined by multiplex PCR. The fitness cost of each of the selected isolates was determined in a competition assay with a reference isolate. The relative fitness was calculated based on the growth of the isolate compared to the reference iso-late.

Results: The mean fitness cost of the 20 S. aureus isolates was –3.4% (SE=1.05). A significant negative correlation between number of antibiotic resistances and fitness of the bacteria was found (R2=0.65; P=0.038). These findings indicate that the isolates are burdened over time as well as by the number of resistances they carry. All tetracycline resistance was carried tet(T) and tet(K), while erm(A) was detected in all erythromycin resistant strains, indicating that the observed difference in fitness is not caused by different resistance markers.

Conclusion: We suggest that increasing fitness cost was an important contribution to the disappearance of multiply resistant MRSA of phage complex 83A during the epidemic in 1965–1975.

Trends in the incidence of methicillin-resistant Staphylococcus aureus nosocomial bloodstream infections in a tertiary care hospital in Greece: a three-year study


Objective: Greece is one of the European countries that experiences high rates of methicillin-resistant Staphylococcus aureus (MRSA). However, data regarding the incidence of MRSA infections are limited. We describe the trends in the incidence of MRSA nosocomial bloodstream infections (BSI) in a large tertiary care hospital in the broad region of Piraeus, Greece.

Methods: Data from January 2006 to December 2008 were analyzed. We focused in hospital departments of high risk for nosocomial infections (ICU, medical and surgical departments). Only the first BSI of each patient was included in the incidence calculation. Staphylocoecal bacteremia was detected by BacT/Alert 3D system (Biomerieux, France); identification and MICs were performed using Vitek 2 (Biomerieux, France) and API Staph systems. Disc diffusion and E-test methods were used to confirm MRSA detection according to CLSI guidelines. The incidence rate of nosocomial BSI due to MRSA was calculated as the number of cases/100 admissions and the number of cases/1,000 patient-days.

Results: Data from 23,107 patients were evaluated. MRSA caused BSI in a total of 44 patients. The incidence rate increased significantly in ICU (from 0.60 cases/100 ICU admissions in 2006 to 1.15 cases in 2007 and to 1.22 cases in 2008 and from 0.57 cases/1,000 patient-days in 2006 to 0.93 in 2007 and to 1.13 cases in 2008; x2 test for trend; P<0.001, respectively). In medical departments the incidence rate was not considerably changed (0.15 cases/100 admissions in 2006, 0.16 in 2007 and 0.21 in 2008), whereas in surgical departments a significant increase was detected (from 0.04 cases/100 admissions in 2006 to 0.13 in 2007 and to 0.17 in 2008). Overall, the total incidence of MRSA increased significantly (from 0.79 cases/100 admissions in 2006 to 1.44 cases in 2007 and to 1.60 cases in 2008 and from 0.89 cases/1,000 patient-days in 2006 to 1.34 in 2007 and to 1.71 in 2008).

Conclusions: In hospital departments with high risk of BSIs, MRSA is a serious concern causing difficulties in treatment and infection control measures. The incidence of nosocomial BSI due to MRSA should be an indicator reflecting the capability of a hospital to control its bacterial ecology using preventive measures against cross-transmission. These preliminary findings suggest that although Gram-negative BSIs prevail in Greece, there is also a raise in the incidence of MRSA bacteraemia. Further analyses are needed to confirm this trend.

Prevalence of Staphylococcus aureus in health professionals in a Brazilian teaching hospital

E. Gir*, M. Carvalho, M. Hayashida, A. Silva, C. Barbosa, S. Canini, S. Santiago, F. Pimenta (Ribeirão Preto, Santo André, Goiânia, BR)

Objective: To analyze the prevalence of Staphylococcus aureus in the saliva of health professionals, in a Brazilian large sized teaching hospital.

Methods: This cross-sectional study was carried out in a large sized public hospital, in Santo André-SP, Brazil between 2006 and 2008, by applying a questionnaire to the participants and collecting saliva in three different moments. Among the 340 participants, 22 were medical doctors, 42 nurses, 99 nursing technicians and 177 nursing auxiliaries. Ethical aspects were considered. After the isolation and identification of the Staphylococcus aureus, the antimicrobial susceptibility tests were carried out. Data were processed using the Statistical Package Social Science (SPSS), version 15.0 for Windows.

Results: Among the 340 professionals who had three saliva samples collected, 162 (47.6%) were colonised. Therefore, the prevalence of methicillin sensible Staphylococcus aureus was of 47.6% (162/340), being 43.5% of methicillin sensible Staphylococcus aureus. Through the disk diffusion test and Etest®, 14 Staphylococcus aureus methicillin resistant (MRSA) were detected (prevalence = 4.1%), being 9 in nursing auxiliaries and 5 in nursing technicians; 11 females and most (42.9%) aged between 19 and 29 years. Regarding the working area, the results were: surgical unit (4), Obstetric centre and Delivery room (4), nursery and Neonatal Intensive Care Unit (2), Intensive Care Unit (ICU) (2), Surgery centre and Post anaesthetical Unit (1), Coronary and Paediatric ICU (1). The MRSA prevalence was of 4.1% (14/340). The MRSA isolated presented 100% resistance to oxacillin; 92.8% to erythromycin, 57.1% to clindamycin, 57.1% to cefoxitin, 42.8% to ciprofloxacin, 7.1% to gentamicin and 7.1% sulfamethoxazole-trimethoprim. All of them presented sensitivity to tetracycline, rifampicin, vancomycin, linezolid and mupirocin.

Conclusion: The prevalence of Staphylococcus aureus among health professionals was of 47.6% (MSSA=43.5%, MRSA = 4.1%). Evaluating the prevalence of MRSA among health professionals is relevant and it also is a preventive measure for hospital infection. It should be invested in preventive and control measures, specially standard precautions and contact precautions, to control the situation of prevalence rates.
Emergence of Panton-Valentine leukocidin-positive Community-onset versus nosocomial bloodstream infections of Staphylococcus aureus

A voluntary national weekly prevalence survey of meticillin resistant reviewed the first six months of the project. Within acute Irish hospitals commenced in April 2008. This paper reviews the first six months of the project.

Methods: The Health Protection Surveillance Centre (HPSC) coordinates data collation and produces quarterly reports for participants. Participants complete an annual baseline form detailing bed capacity and isolation room facilities and weekly MRSA surveillance data on the same day each week.

Results: Thirty-two ICUs submitted data during the first two quarters of the project (22 general, 9 regional and one specialist hospital). Nineteen ICUs contained a mixture of ICU and high dependency/cornerly care patients (mixed ICUs) and 13 contained ICU patients only (non-mixed ICUs). The majority (20) contained between five and ten beds, with nine containing less than four beds. Four have no rooms available for isolation. Of the remainder, the average number of rooms is 2.2 rooms per ICU, only eight ICUs have three or more isolation rooms, of which 39% have an anteroom and 76% a handsink. All ICUs screen patients for MRSA on admission. The median rate of ventilated patients was 44.2% (range 1.6–82.5%), with a higher rate in non mixed (0–82%, median 54.7%) than mixed ICUs (1.6–76%, median 25.6%). National MRSA ICU prevalence rates ranged from 0% to 25% (median 8.7%) and were higher in non-mixed (median 13.5%) than mixed ICUs (median 7.1%). MRSA acquisition ranged from 0% to 3.7% (median 0.2%) and again was higher in non mixed ICUs (median 0.6%).

Conclusions: Isolation room facilities are crucial for the prevention and control of MRSA. There is a wide variation in isolation room facilities across ICU’s. Many of the single rooms cannot be truly classified as isolation rooms as they lack an isolation room such as a hand sink and anteroom. Since ICUs vary considerably in size, the provision of isolation rooms, direct comparisons between ICUs is difficult. However, data can be used locally to monitor trends over time. A more detailed study is required to more accurately identify the underlying issues within each hospital.

P1869 Surveillance of methicillin-resistant Staphylococcus aureus within Irish intensive care units


A voluntary national weekly prevalence survey of meticillin resistant Staphylococcus aureus (MRSA) in general intensive care units (ICUs) within acute Irish hospitals commenced in April 2008. This paper reviews the first six months of the project.

Methods: The world wide emergence of CA-MRSA infections caused by hypervirulent strains producing PV leukocidin (PVL, coded lukS-lukF) is a problem of extreme interest. Molecular studies suggest a spread of a limited number PVL-producing MRSA clones that are genetically distinct from hospital-acquired strains. This emergence of PVL+ CA-MRSA represents a public health threat, because these strains are distinct from hospital-acquired strains. This emergence of PVL+ CA-MRSA/MSSA represents a priority even in small hospitals.

P1870 Community-onset versus nosocomial bloodstream infections due to meticillin-resistant Staphylococcus aureus in Spain

A.B. Millán, M.A. Domínguez, C. Borras, M.P. González, B. Almirante, E. Cercenado, B. Pujilla, M. Pujol, J. Rodríguez-Baño* and GEIH/GEMARA/REIPI

Objectives: Community strains of methicillin-resistant Staphylococcus aureus (MRSA) are still rare in Spain, but a significant proportion of infections caused by MRSA are community-onset, healthcare-associated. The aims of this study were to compare the epidemiology and clinical features of community-onset and nosocomial bloodstream infections (BSI) due to MRSA in Spain.

Methods: Prospective cohort of cases of BSI due to MRSA from 59 Spanish hospitals during June 2003. Episodes were considered community-onset when diagnosed within 48 hours of hospital admission, and nosocomial when diagnosed subsequently. Community-onset episodes were subclassified as healthcare-associated on the bases of epidemiological data (Friedman’s criteria) and molecular typing of isolates (e.g., clonal relationship with typical nosocomial isolates), which was performed using PFGE and MLST.

Results: We included 64 episodes; 21 (33%) were community-onset, all of which were considered healthcare-associated. We found no significant differences between community-onset and nosocomial BSI regarding demographic features or clinical and epidemiological characteristics, except for the source of BSI: central venous catheter was more common among nosocomial episodes (39% vs 5%, p = 0.005), while the urinary tract was more common among community-onset episodes (25% vs 0%, p = 0.001). Empirical treatment was inappropriate in 86% of community-onset episodes and in 67% of nosocomial episodes (p = 0.1). Related mortality and 30-day mortality were 19% vs 23% (p = 0.7), and 19% vs 28% (p = 0.4), respectively.

Conclusions: One third of BSI due to MRSA bacteraemia in our study was considered community-onset, and all of them were healthcare-associated. The epidemiological and clinical features of community-onset and nosocomial episodes were similar, except for the sources of
bacteraemia. Clinicians should be aware of the need to consider coverage against MRSA more frequently, particularly for certain infectious syndromes in patients with community sepsis and previous healthcare association.

**P1871** Regional variations of methicillin-resistant *Staphylococcus aureus* incidence densities among 169 German hospitals which participated in the MRSA-KISS module

I.F. Chaberny*, F. Schwab, P. Gastmeier (Hanover, Berlin, DE)

**Background:** A country-wide prospective multicentre hospital-based surveillance of MRSA case-patients was established in the year 2003 with participating hospitals of the national German nosocomial infections surveillances system (KISS). This is called the MRSA-KISS module.

**Objectives:** To assess regional variations of MRSA incidence densities in German hospitals.

**Methods:** The data were recorded during routine surveillance by the infection control team of each hospital and send to the national reference centre for analysing the data. The German Federal Counties were grouped into five regions to create comparable regions according to the number of inhabitants (North (N), West (W), East (E), South-West (SW) and South-East (SE)). The summarised data from 2006 and 2007 were stratified to the five regions and a multiple logistic regression was performed.

**Results:** A total of 36,162 MRSA case-patients and 36,797,125 patient days from 169 hospitals were analysed. MRSA identification later than 48 h after admission was classified into nosocomial cases. Hence, 28.1% (24.7% to 31.7%) were nosocomial and a total of 71.9% (68.3% to 75.3%) were imported MRSA case-patients. The total MRSA incidence density was 0.98. The data show significant differences of the regions (see Table 1).

**Conclusion:** This study demonstrated significant regional variations according to the MRSA incidence densities, which may be explained by differences of the various dominating MRSA strains or variations of the infection control habits among the regions.

**Table 1:** MRSA incidence densities (ID) (MRSA case-patients per 1,000 patient days) of 169 hospitals grouped into 5 regions

<table>
<thead>
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<th>Region</th>
<th>ID (pooled mean)</th>
<th>East</th>
<th>South-East</th>
<th>South-West</th>
<th>North</th>
<th>West</th>
<th>Total</th>
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<td>ID (pooled mean)</td>
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<td>SCCmec (mosaic)</td>
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<td>1.04</td>
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**P1873** Epidemiology of MRSA bacteraemia and clinical relevance of reduced susceptibility to vancomycin

T. Lewis, R. Chaudry, I. Das*, P. Lambert (Birmingham, UK)

**Objectives:** Recent reports suggest a reduced efficacy of vancomycin in the treatment of meticillin resistant *Staphylococcus aureus* (MRSA) infection. This has been attributed to a reduced susceptibility of MRSA to vancomycin. We analysed the epidemiology and outcome of MRSA bacteraemia. We determined the minimum inhibitory concentrations (MIC) of vancomycin and other antibiotics, and looked for the presence of heteroresistant vancomycin intermediate *Staphylococcus aureus* (hVISA). We looked for associations of raised vancomycin MIC and hVISA with clinical outcome.

**Methods:** University Hospital Birmingham is a 1200 bed tertiary referral centre. Episodes of MRSA bacteraemia over a 28 month period in 2005–7 were retrospectively analysed. hVISA were identified by the presence of microcolonies at ≥6 mg/l using the macro E-test method. SCCmec typing and sub-typing was carried out by PCR.

**Results:** We identified 195 distinct episodes of MRSA bacteraemia from 179 patients, which included 16 relapses. 194 episodes were healthcare-associated infections. Intravascular devices were the most common focus of infection (39%). Overall mortality at 30 days was 28%. Mortality was highest in those with no identifiable focus (52%), compared with all other sites (17%; p < 0.01). The modal vancomycin MIC was 0.5 mg/l, and no isolates had a vancomycin MIC above 1.5 mg/l. All isolates were sensitive to linezolid, daptomycin and tigecycline. 18% of isolates were hVISA. hVISA was associated with previous vancomycin usage (p = 0.02) and specialties that use high volumes of vancomycin (p = 0.01). We did not observe an association of hVISA with high burden sources. Compared to non-hVISA episodes, hVISA was not associated with increased mortality (p = 0.1), relapse rate (p = 0.48) or rate of other complications (p = 0.56). SCCmec type IVa (EMRSA15) was the most common type in both hVISA and non-hVISA groups.

**Conclusions:** This is the first report analysing the prevalence and clinical relevance of hVISA from the UK. We identified a high prevalence of vancomycin heteroresistance. This is associated with vancomycin use, both in individual patients and in particular specialties, suggesting antibiotic selection pressure is changing the ecology of MRSA. There was no correlation between hVISA and adverse outcome. MRSA bacteraemia remains a serious infection with a high mortality and high rate of relapse. Further studies are required into the epidemiology and continued evolution of hVISA.

**P1874** Detection and characterisation of MRSA 6–12 months post successful decolonisation: persistence or re-colonisation?

D. Gilpin*, S. Small, S. Bukkshi, P. Kearny, M. Tunney (Belfast, Antrim, UK)

**Objectives:** Colonisation by Meticillin-resistant *Staphylococcus aureus* (MRSA) is considered to be the pre-cursor to invasive infection. It is widely accepted that decolonisation, using topical agents such as chlorhexidine and mupirocin, is key to reducing the transmission and MSSA isolates, none was found to carry the PVL genes, while one strain was positive for TST-1 gene and five for the enterotoxin gene cluster. All MRSA isolates were susceptible to Linezolid, Quinupristin/dalfopristin, Cotrimoxazole but resistant to ciprofloxacin and 4/13 resistant to Rifampin.

**Conclusions:** 1. The prevalence of MRSA strains among CF adult patients found to be 8.6%, in agreement with the European records. 2. Although PVL coding gene was present only in one strain, the finding suggested an urgency for our CF population, because of necrotising pneumonia fear. 3. The presence of TST-1 gene as well as enterotoxin genes in both MRSA and MSSA isolates imposes the systemic staphylococcal gene toxins determination for early treatment of CF patients harbouring toxigenic strains in their respiratory system.
spread of MRSA within the hospital environment. This study aimed to re-screen successfully decolonised patients at 6 and 12 months to determine the extent of persistence, or re-colonisation, of MRSA among these patients.

Methods: MRSA-colonised patients were identified by routine sample submission to the diagnostic laboratory, at Antrim Area Hospital and decolonised in accordance with standard hospital protocols (4% (w/v) chlorhexidine wash once daily, and mupirocin applied nasally three times daily, for seven days). Patients were described as “decolonised” after submission of three negative screening swabs, each one-week apart. At 6 and 12 months, the patients were re-screened. Pre- and post-decolonisation isolates were analyzed by pulsed-field gel electrophoresis (PFGE) and similarities between banding patterns obtained compared using GelCompar II.

Results: Of 92 successfully decolonised patients, 36 (39.1%) were positive at either 6 or 12 months post decolonisation. Post-decolonisation isolates were obtained for 25 patients. Of these, isolates from 21 patients were similar or identical to pre-decolonisation isolates (>70% homology). Isolates from the remaining 4 patients shared <60% homology pre- and post-decolonisation and were recorded as different.

Conclusion: This study suggests the need for 12 month follow up screening for successfully decolonised patients. For the majority of patients, standard decolonisation protocols were found to be effective for long-term (up to 12 months) decolonisation of MRSA. In a small number of cases, patients were re-colonised with a different strain of MRSA. However, in a significant proportion of patients, despite being apparently effectively decolonised, the same strain, as was initially isolated, was detected up to 12 months post decolonisation. This may be due to a range of factors including repeated exposure to the same source of MRSA or a reduction in MRSA numbers to below detectable level without complete eradication. Further phenotypic analysis of these isolates is currently underway, together with more detailed examination of patient characteristics.

Risk factors for methicillin-resistant Staphylococcus aureus

B. Catry, E. Hendrickx, R. Preal, R. Mortens (Brussels, BE)

Objectives: The present study aimed to investigate the relationship between the prescriptions of antimicrobial agents and infection/colonisation with MRSA in the ambulatory and in the in-patient setting.

Methods: The microbiological results retrieved from 16 voluntary participating clinical laboratories during 2005 were coupled with the individual antimicrobial consumption patterns (July 2004-December 2005) as provided by the pooled data of the seven Belgian health insurance funds (intermutualistic agency, IMA). Herein, all Staphylococcus aureus positive patients (only first isolate) and susceptibility testing result for oxacillin were retained. Logistic regression was used to identify risk factors for oxacillin resistance (MRSA) following antimicrobial consumption and sociodemographic characteristics (e.g. age, admission to healthcare setting, ...). Antimicrobial consumption was transformed into defined daily doses (DDD) and categorised using the ATC classification up to 4 digits (eg. J01C) according to WHO terminology (2007).

Results: A total of 6844 patients were included in the final logistic regression model, of which 1200 (17.5%) died in the year 2005. Within the latter group, 51.2% (n=614) were MRSA positive whereas in the patients that survived 2005 only 28.1% (1568/5644) were found to have MRSA (OR for death in case of MRSA=2.68; 95% CI 2.36–3.05; p<0.01). The multivariate model found following factors (85% C-statistics to be significant (p<0.01) associated with MRSA: admission to a long term care settings (3.22–5.11); aged 55–104 (3.76–6.35); aged 15–54 (1.36–2.37); consumption (per DDD) of cholinolones (1.017–1.026); cephalosporins, monobactams and carbapenems (1.004–1.012); penicillins, aminopenicillins +/- clavulanic acid, [beta-lactamase stable penicillins (1.0066–1.0039)].

Conclusions: These data strongly support the existence of a risk for acquired antimicrobial resistance in the major bacterial pathogen Staphylococcus aureus, directly related to the consumption of antimicrobial agents at the individual patient level. In addition the study confirmed an association of MRSA with specific healthcare settings and age.
residents were swabbed, and a urine culture was obtained in the presence of a urinary catheter. Incidence of MRSA infection (defined according to CDC criteria) in carriers was evaluated with a questionnaire sent every 3 months to nurses from 2005 to 2008. Standard precautions were applied for all NI's residents including MRSA carriers (except transmission-based precautions for colonised or infected wound or urine).

Results: The proportion of MRSA carriers was 4.5% (39/872 residents) in 2003, 10.3% (179/1730) in 2005, and 12.0% (273/2275) in 2008 (p < 0.001). Among them, 34 (87%), 116 (65%), 112 (41%) respectively, were newly identified. A total of 413 residents participate in both the 2005 and 2008 surveys: among the 60 of them who were MRSA carriers in 2005, 35 (58%) were no longer screened positive in 2008.

Conclusions: We observed a 167% increase (up to 12%) in prevalence of MRSA carriage over 5 years in NI’s residents of Western Switzerland. Although the estimated risk of infection was moderate (0.1 episode/resident-year), this trend should prompt reappraisal of the infection control measures for MRSA carriers in this setting.

P1879 Staphylococcus aureus colonisation/infection in a neonatal intensive care unit: a four-year study

M. Papadimitriou*, E. Koemtzidou, G. Antonaki, M. Matsas, A. Alexi, G. Kourakis, E. Lebessi (Athens, GR)

Objectives: To assess the colonisation/infection by Staphylococcus aureus among neonates in a 30-bed, university-affiliated, level III-IV Neonatal Intensive Care Unit (NICU) at a large paediatric hospital in Athens.

Methods: All cases of S. aureus infection or colonisation in the NICU were identified by using the data from laboratory and the medical records. Routine surveillance cultures for the detection of multidrug resistant pathogens was a standard practice in our NICU. Surveillance consisted of swabbing the throat and rectum upon admission and weekly until discharge. When infection is suspected, additional cultures are taken (blood, urine, stool, skin lesions, umbilical and eye swabs). Culture of samples and identification was made by standard methods. Susceptibility to penicillin (PN), oxacillin (OX), cefoxitin (FOX), kanamycin (KN), tobramycin (TB), gentamicin (GN), erythromycin (ER), clindamycin (CL), ciprofloxacin (CP), fusidic acid (FA), trimethoprim-sulphamethoxazole (SXT), tetracycline (TE), rifampin (RF), chloramphenicol (CHL), vancomycin (VAN) and teicoplanin (TEC) was tested using the disk diffusion method, according to the current CLSI guidelines.

Results: During the study period (2004–2007), 1822 neonates (59% male and 41% female) were admitted in the NICU. Two hundred seventy neonates (14.8%), ranging in age from 3 to 54 days, were found colonised with S. aureus upon admission (referred cases), whereas 69 neonates (3.8%) were colonised during their NICU stay (NICU-acquired cases). The median length of stay to the NICU before colonisation was 14 days (range 3–207 days). The incidence of methicillin resistant S. aureus (MRSA) was 21.8% (59/270) and 23.1% (16/69) among referred and NICU-acquired cases, respectively. Yearly incidence of MRSA isolates from 2004 through 2007 was as follows: 17.1%, 21.2%, 28.0%, and 22.2%. The following resistance phenotypes were identified: PN/OX (33.8%); PN/OX/FA/KN/TE (14.9%); PN/OX/TB/KN (9.5%). Infection due to S. aureus was identified in 29 cases (bacteremia, 3; UTI, 2; ophthalmia, 18; cutaneous infections, 3; umbilicities, 3), and to MRSA in 17 cases (ophthalmia, 8; cutaneous infections, 7; umbilicities, 2). Epidemics were not identified.

Conclusions: S. aureus appears endemic in maternity units and NICUs. The rate of MRSA is very high. Systematic surveillance to optimise detection of colonised newborns and aggressive infection-control measures in maternity units and NICUs are necessary to prevent the spread of MRSA.

P1879 Frequency of mecA gene and borderline oxacillin resistant Staphylococcus aureus in nosocomial acquired methicillin resistance Staphylococcus aureus infections

F. Khorvash, K. Mostafazadeh, S. Mobasherizadeh, M. Jalali* (Isfahan, IR)

Objectives: MRSA (methicillin resistant staphylococcus aureus), one of the most important causes of nosocomial infections, is now endemic in many hospitals. The aim of the study was to determine the frequency and type of MRSA strains and antibiotic susceptibility in Al-Zahra Hospital, Isfahan, Iran.

Methods: In an analytic descriptive survey in 2005 and early 2006, patients admitted to the hospital who contracted S. aureus nosocomial infections were enrolled in the study. All isolates were identified by the conventional laboratory tests. MIC (Minimal Inhibitory Concentration) of oxacillin on isolated bacteria was determined by E-Test method. According to CLSI (Clinical and Laboratory Standard Institute) criteria all strains with MIC of $\geq 4 \mu g$ for oxacillin were identified as MRSA.

Intrinsic high level resistance (Mec A positive) and borderline oxacillin resistant staphylococcus aureus (BORSAs) were detected by amoxicillin-clavulanate E-Test strain. Strains with MIC of $\geq 8 \mu g$ for oxacillin and $\geq 32 \mu g$ for amoxicillin-clavulanate were identified as mec A negative MRSA (BORSAs) MIC of vancomycin also was determined on isolated bacteria. Data were analyzed by SPSS version 13 and Who net version 5.

Results: Out of 134 Staphylococcus aureus samples which were isolated from nosocomial infections 90(67.2%) were MRSA. 67 out of 90(74.5%) were mec A positive MRSA. Other staphylococcus with MIC $\geq 4\mu g$ for oxacillin and $\leq 8 \mu g$ for amoxicillin-clavulanate were identified as mec A positive MRSA. Other staphylococcus with MIC $\geq 4 \mu g$ for oxacillin and $\leq 8 \mu g$ for amoxicillin-clavulanate were identified as mec A negative MRSA (BORSAs). MIC of vancomycin was also determined on isolated bacteria.

Conclusion: Because one fourth of our staphylococcus strains are mec A negative BORSAs and there is no alternative for vancomycin against mec A positive MRSA and Enterococcus spp. in our hospital, vancomycin should be reserved only for life threatening infections due to these organisms. Thus MRSA typing should be done to choose appropriate antibiotic for optimal treatment of MRSA infections.

P1880 A four-year trend of in vitro sensitivity profile of Staphylococcus aureus strains cultured at a teaching hospital, northern Italy

R. Manfreidi*, A. Nanetti (Bologna, IT)

Introduction: The increased rate of drug resistance among Gram-positive cocci is a general concern, especially in hospital settings. A prospective bacteriological monitoring including a continued surveillance of antimicrobial susceptibility rates, is ongoing at our General Hospital, since the year 2004.

Materials and Methods: The temporal variations of in vitro antimicrobial sensitivity figures were examined quarterly for all suitable Staphylococcus aureus strains, and followed from 2004 to 2007. The same pathogen cultured more than once from the same patient within one month, has been considered once.

Results: Among overall Staphylococcus aureus isolates (1,863 strains tested on the whole), a complete (100%) sensitivity was shown against vancomycin and teicoplanin, while some compounds retained interesting activity (92.0–97.1% for cotrimoxazole, 76.1–88.7% for chloramphenicol, 64.1–69.5% for rifampin). Oxacillin (methicillin) resistance ranged from 46.2% of year 2007, to 53.3% of year 2008.
Assessment of screening all patients and the environment occurrence of methicillin-resistant Staphylococcus aureus is important, to found reliable guidelines of antibiotic treatment and prophylaxis, in common clinical settings.

Despite a stable, significant rate of methicillin resistance (mean value around 47% of all Staphylococcus aureus isolates), we have to underline that “older” compounds like cotrimoxazole, chloramphenicol, and also rifampin, may still play some role in selected clinical situations, while the activity of available glycopeptides is 100% maintained presently.

Conclusions: A long-term bacteriological surveillance of antimicrobial susceptibility rates of relevant hospital-related microorganism like Staphylococcus aureus is important, to found reliable guidelines of antibiotic treatment and prophylaxis, in common clinical settings.

Method: In June 2008, a nasal swab was obtained from patients presenting to the Emergency Department, and it was cultured in nutrient broth and tested for the presence of MRSA using standard laboratory methods. Patients answered a standardised questionnaire regarding risk factors for MRSA colonisation. CDC epidemiological definitions for CA-MRSA were used. Ethical permission was received from the Hospital’s Ethics Committee.

Results: 538 patients were included in the study; two patients withdrew from the study prior to obtaining the nasal swab. MRSA was recovered from nasal swabs of fifteen patients (2.8%). Five of the fifteen patients fulfilled the definition for CA-MRSA, hence the prevalence of CA-MRSA was 0.93%. Only one isolate was ciprofloxacin susceptible and 6 isolates (40%) were fucidin resistant. No vancomycin intermediate Staphylococcus aureus (VISA) was detected. The median age of patients was 42 years (inter-quartile range 28–68 years) and 57.8% patients were male. Almost half (45.4%) of patients were from the immediate surrounding area of the hospital.

On bivariate analysis; living alone, age sixty years or over, having in long term care, attending the General Practitioner in the last year, presenting with a medical/surgical complaint (in comparison to a musculoskeletal or psychiatric complaint) and having chronic obstructive pulmonary disease (COPD) were all found to be statistically significant (p < 0.05). A multivariate analysis was then performed to ensure that there was no bias effect amongst the risk factors. Multivariate analysis indicated that, aged sixty years or over (p = 0.01), having COPD (p < 0.01) and presenting with a medical/surgical complaint (p = 0.03) were independent risk factors.

Conclusions: Prevalence of CA-MRSA in this region of Dublin, Ireland is low (1%). At present, limiting MRSA screening to patients with specific risk factors may represent a more efficient use of resources for our patient population.

### Table 1: Resistance against oxacillin in different specimens with various phenotypic and genotypic tests

<table>
<thead>
<tr>
<th>Sample</th>
<th>Resistant</th>
<th>Disk diffusion</th>
<th>mecA gene positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (n=60)</td>
<td>29 (48%)</td>
<td>30 (50%)</td>
<td>30 (50%)</td>
</tr>
<tr>
<td>Urine (n=37)</td>
<td>16 (43%)</td>
<td>16 (43%)</td>
<td>17 (46%)</td>
</tr>
<tr>
<td>Cutaneous sample (n=43)</td>
<td>18 (42%)</td>
<td>18 (42%)</td>
<td>19 (44%)</td>
</tr>
<tr>
<td>Respiratory tract (n=55)</td>
<td>27 (49%)</td>
<td>27 (49%)</td>
<td>28 (51%)</td>
</tr>
<tr>
<td>Other (n=9)</td>
<td>4 (45%)</td>
<td>5 (46%)</td>
<td>5 (46%)</td>
</tr>
<tr>
<td>Nasal swab (n=31)</td>
<td>11 (35%)</td>
<td>12 (39%)</td>
<td>11 (35%)</td>
</tr>
</tbody>
</table>

*According to NCCLS guidelines isolates with MIC ≥ 4 μg are resistant to oxacillin.
Antimicrobial susceptibility in PVL positives Methicillin-resistant Staphylococcus aureus

Table. Number of strains and percentages of resistance

<table>
<thead>
<tr>
<th>Resistance</th>
<th>OXA</th>
<th>ERY</th>
<th>CLI</th>
<th>GEN</th>
<th>CIP</th>
<th>RIF</th>
<th>SXT</th>
<th>VAN</th>
<th>DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>29</td>
<td>18</td>
<td>7</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Percentage</td>
<td>64.44%</td>
<td>40%</td>
<td>15.55%</td>
<td>0%</td>
<td>20%</td>
<td>2.22%</td>
<td>2.22%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Conclusion: our results show good correlation between phenotypic and genotypic methods for antibiotic susceptibility tests. Highest percent of MRSA strains isolated from respiratory tract samples (%49), followed by Blood (%48), other samples (for example Tissues or Excudates or Bone marrow) (%45), Urine (%43), Coetaneous samples (%41) and finally Nasal swabs (%34). These differences were not statistically significant (P > 0.05).

P1885 Staphylococcus aureus methicillin-resistant colonisation among healthcare workers at a general hospital

Objectives: Studies of MRSA colonisation among health care workers (HCW) are important in order to control the emergence and spread of this organism in health-care institutions. The goal of the study was to estimate the prevalence of MRSA colonisation in HCW.

Methods: A total of 67 HCW were studied from January-February 2007, a nasal swab was obtained from each participant in the study. The nasal swabs were cultured on MRSA ID media (bioMérieux). The cultures were read at 24 and 48 hours. The MRSA phenotype was confirmed by the identification of the mecA by PCR. The genetic relatedness of the isolates was studied by RFLP (cfo I)-PCR of the coa gene. The spa, MLST, SCCmec, and agr types were studied. The presence of the pvl genes were studied by PCR.

Results: Six HCW were colonised by MRSA strains (8.9%). The strains according to the RFLP (cfo I)-PCR, were identified as two clones. The isolates belong to the 008-ST38-IV-agr 1 (CC8) (n=2), and 018-ST36-II-agr 3 (CC30) (n=4). Both clones were PVL negative.

Conclusion: The colonisation of HCWs with the epidemic community USA 300 related clone (008-ST38-IV-agr1) is a matter of concern. The other clone belongs to the international British EMRSA-16 (018-ST36-III-agr 3) that is endemic in the studied hospital. HCWs could be the reservoir for the spread MRSA strains.

P1886 Methicillin-resistant St. aureus infections among patients in an emergency department
VR. Bollineni* (Kharkov, UA)

Background: Methicillin-resistant Staphylococcus aureus (MRSA) is increasingly recognized in infections among persons in the community without established risk factors for MRSA.

Methods: We enrolled adult patients with acute, purulent skin and soft-tissue infections presenting to 11 university-affiliated emergency departments during the month of August 2004. Cultures were obtained, and clinical information was collected. Available S. aureus isolates were characterised by antimicrobial-susceptibility testing, pulsed-field gel electrophoresis, and detection of toxin genes. On MRSA isolates, we performed typing of the staphylococcal cassette chromosome mec (SCCmec), the genetic element that carries the mecA gene encoding methicillin resistance.

Results: S. aureus was isolated from 320 of 422 patients with skin and soft-tissue infections (76 percent). The prevalence of MRSA was 59 percent overall and ranged from 15 to 74 percent. Pulsed-field type USA300 isolates accounted for 97 percent of MRSA isolates; 74 percent of these were a single strain (USA300-0114). SCCmec type IV and the Panton-Valentine leukocidin toxin gene were detected in 98 percent of MRSA isolates. Other toxin genes were detected rarely. Among the MRSA isolates, 95 percent were susceptible to clindamycin, 6 percent to erythromycin, 60 percent to fluoroquinolones, 100 percent to rifampin and trimethoprim-sulfamethoxazole, and 92 percent to tetracycline. Antibiotic therapy was not concordant with the results of susceptibility testing in 100 of 175 patients with MRSA infection who received antibiotics (57 percent). Among methicillin-susceptible S. aureus isolates, 31 percent were USA300 and 42 percent contained pvl genes.

Conclusions: MRSA is the most common identifiable cause of skin and soft-tissue infections among patients presenting to emergency departments in 11 U.S. cities. When antimicrobial therapy is indicated for the treatment of skin and soft-tissue infections, clinicians should consider obtaining cultures and modifying empirical therapy to provide MRSA coverage.
Costs and benefits of a peri-operative screen-and-treat strategy in nasal carriers of *S. aureus*

M.M.L. van Rijen*, L.G.M. Bode, M.C. Voë, J.A.J.W. Kluytmans for the STEP Study Group

Objectives: A multicentre double-blind randomised-controlled trial (M-RCT), carried out in the Netherlands between October 2005 and June 2007, showed that hospitalised patients with *S. aureus* nasal carriage who were treated with mupirocin nasal ointment and chlorhexidine gluconate medicated soap (MUP-CHX), had a significantly lower risk of nosocomial *S. aureus* infections than patients receiving placebo (3.4% vs. 7.7%, 95% CI 0.23–0.75). We determined the costs and benefits of MUP-CHX in patients undergoing elective surgery.

Methods: The costs consisted of the screening and the treatment costs. To estimate the benefits, the difference in length of stay (LOS) between the two treatment groups was determined and the associated costs were estimated.

Results: In total, 5736 patients were screened pre-operatively. 1062 of these patients were found to be nasal carrier of *S. aureus*. Subsequently, 799 of them were included in the M-RCT: 436 in the MUP-CHX group and 363 in the placebo group. To identify one carrier, 6 patients had to be screened. The cost of one screening test was €25, resulting in €150 to identify one carrier. The costs of treatment with MUP-CHX were €13 per carrier. So, the total costs were €163 per treated carrier. Costs for one patient day were €385. The mean LOS was 2.2 days shorter for patients treated with MUP-CHX (11.8 vs. 14.0, p = 0.032), resulting in a savings of €847. Taking into account the screening and treatment costs and the savings for prevented LOS, €684 was saved per MUP-CHX treated patient. Subgroup analysis revealed that the strategy was most cost-effective in cardiothoracic surgery.

Conclusion: Screening for *S. aureus* carriage in elective surgical patients and subsequently treating carriers with MUP-CHX is highly cost-effective.

**P1889** Carriage of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in medical microbiology laboratory personnel

M. Jager*, J. Kluytmans, C. Vandenhoutte-Grunds (Amsterdam, NL)

Objective: To determine whether personnel working in clinical microbiology laboratories in The Netherlands is at increased risk for colonisation with MRSA.

Methods: Anterior nares and throat swab samples were obtained from 266 employees of five different clinical microbiology laboratories. Both swabs were placed in enrichment broth (Becton, Dickinson and Company, France); these were incubated for 48 hours at 37°C and then subcultured by plating aliquots on a chromogenic selective medium (bioMerieux, France) for 24 hours at 37°C for the isolation of MRSA, and on a mannitol salt agar for 48 hours at 37°C for the isolation of *S. aureus*. MRSA and *S. aureus* were identified according to standard procedures for each participating individual; the professional category (technician, clinical microbiologist, trainee or other) was noted.

Results: *S. aureus* was detected in 45.1% (CI 95%: 39–51) of the screened individuals. Of these, 31.7% (CI 95%; 20–50) carried *S. aureus* in the throat only. MRSA was detected in the throat sample of one person (0.38%; CI 95%: 0.07–2.11). Further analyses of the MRSA strains isolated in that laboratory revealed that the employee who carried the MRSA strain worked with the same clinical strain months before. AFLP was used, to confirm that the strains were similar. There was no significant difference in carriage rate of *S. aureus* between the five laboratories nor between employees of different professional categories.

Conclusions: To the best of our knowledge, this is the first assessment of carriage of *S. aureus* and MRSA in a microbiology laboratory. We found a high carriage rate of *S. aureus*; a reason for this could be that both nose and throat samples were used for carriage detection. One employee carried a MRSA strain that had been recently manipulated in the laboratory. Therefore, it may indicate occupational acquisition of MRSA. The finding that 0.38% of lab personnel is MRSA positive is not significantly different from a previous survey of patients on admission to the hospital in The Netherlands. Therefore, we conclude that risk of acquiring MRSA during work in a microbiology laboratory is limited.

**P1890** Exploring the fourth dimension: the clinico-economic impact of a distinct model of MRSA screening by PCR in United Kingdom

A. Galeri, R. Palmer, C. Danison, B. Lunt, S. Ellershaw*, J. Lickiss, J. Carter Lindsay, K. Woodrow, N. Harper (Blackpool, UK)

Background: Accurate and rapid identification of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospital admissions is essential for timely decisions on isolation/bio-burden reduction, effective antimicrobials if infected, and reducing the potential for cross transmission and self acquisition of HCAI. Blackpool Victoria Hospital [BVH] is a large district hospital in northwest England housing two speciality centres [cardiac surgery and haematology]. Hospital charitable committee provided grant to pump prime a 6-month pilot using MRSA PCR to screen all emergency admissions [medical and surgical]; intensive care, high dependency and surgical high care unit admissions.
Method: The steering group [Microbiologists, Diagnostics Manager, nursing leads – medicine/surgery, infection control and data analyst] strategy included: clinico-economic modelling; revised MRSA guideline; analysis of hospital admissions/24 hrs; comprehensive MRSA containment strategy [management-infection management team-clinical staff liaison; advertisement & training/education drive; raise awareness; dress code; management run infection prevention road shows; etc]. The project [over 6-months] offered PCR runs 8am–midnight [TAT 3–8 h]. Snap shot audits of TAT [swab to patient isolation], real time data analysis and feeding back.

Results: Total reduction in MRSA infection episodes [bacteraemias 63% and wound infections 38%]; 31% [2865/4145] reduction in estimated glycopeptides unit-days over 6-months [2008 v 2007]and cost savings; enhancing quality care and patient safety. Fourth dimension – MRSA PCR is used in clinical decision making, early institution of optimal therapy/alternate diagnosis and reduction in morbidity/mortality. Cost saving: Estimated savings from [cost of total MRSA infection episodes prevented + savings from reduced GP usage] minus [cost of test + staffing]. First in region/country to use clinico-economic modelling to run 8am–midnight service with demonstrable success.

Conclusions: Following success of the pilot, the trust has accepted a business case for regular delivery of MRSA PCR service. A remarkable reduction in total MRSA infection episodes including bacteraemias. A lack of correlation between high risk and MRSA carriage noted. This overall success is attributed to the comprehensive bundle of initiatives including PCR. The clinical impact of MRSA PCR has been most significant in guiding decision making. A separate study is set to analyse this impact.

Results: Total reduction in MRSA infection episodes – PCR introduced mid March.

Molecular assay for the rapid screening of methicillin-resistant \textit{S. aureus} colonised patients in an intensive care unit

P. Cavallerio *, C. Parlato, L. Fossati, R. Serra (Turin, IT)

Objectives: The prevalence of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is constantly increasing in hospitals and communities in Europe. The costs that MRSA cause on the healthcare system are often underestimated, because of the significant prevalence of asymptomatic carriers colonised. To minimise the time needed for isolation of patients a rapid identification of MRSA colonisation is needed. We used the GeneXpert™, an automated real-time-PCR system and the diagnostic kit, Xpert MRSA (Cepheid, Inc., Sunnyvale, CA) to screen nasal swabs from about 34 patients admitted to and outgoing of an intensive care unit (ICU). The results were compared to culture-based methods to assess sensitivity and specificity in detecting carriers of MRSA. The aim is to propose a new rapid screening method for the intensive care units of our hospital.

Methods: 65 Patients from an ICU unit at San Giovanni Battista Hospital were included in the study. Double nasal swabs (Copan®, Italy) were taken from patients. One was used for amplification directly using Xpert MRSA. The second was for the culture control, that included the enrichment with Tryptic Soy Broth with NaCl 6.5% for 24 h and then inoculated onto agar. Patients with positive tests results were put in isolation and treated, for the decolonisation, with nasal mupirocin and baths of 4% chlorhexidine for 5 days. After eight days a nasal swab control was taken. Patients with negative results were reexamined after seven days.

Results: A total of 122 nasal swabs were tested by using Xpert MRSA assays and culture-based-methods. Nine (7.4%) were unresolved. Nightly-eight specimens (78.7%) were negative and six were positive (4.9%) for MRSA by Xpert MRSA and culture-based-methods. Eleven (9.0%) were positive for MRSA by the PCR assay and, in contrast, were negative by culture-based-methods and classified as false-positive. However, five of these, were positive by PCR-assay but negative by culture-based method, because have been performed after decolonisation.

Conclusion: Xpert MRSA™ detects patients with MRSA nasal colonisation in less than 2 hour and it’s easy to use. Compared to culture-based methods, the Xpert MRSA assay provided significantly faster turnaround times and resulted in more prompt isolation of MRSA-colonised patients. The relevance of samples false positives by the PCR method is to take into account; however, it should be noted that there were no false negative by PCR assay.

Cost saving therapy/alternated diagnosis and reduction in morbidity/mortality.

Methods: Incremental direct costs caused by addition of PCR (BD GeneOhm™ MRSA assay) or chromogenic agar (MRSA-ID by bioMérieux) to conventional cultures were calculated applying a hospital perspective. This included costs attributed to the rapid screening tests, costs because of false negative test results and savings because of avoided isolation days. The number of pre-emptive isolation days avoided with rapid diagnostic testing was determined in a prospective multi-centre experimental cohort study in 12 Dutch hospitals. This study included 853 patients.

Results: Costs of an isolation day, including supply costs and the extra time needed by nurses, physicians and cleaning personnel were estimated to be €27.30. The number of isolation days was reduced by 60.4% with PCR-based screening and would have been reduced with 47.4% with chromogenic agar screening. Cost per test, when added to standard culture procedures, was €52.70 for the PCR and €2.05 for chromogenic agar. Four false negative test results during the study resulted in additional costs of €10,077.41. The costs per isolation day avoided were €92.25 and €8.28 for PCR and chromogenic testing, respectively. Performing MRSA PCR added €143.73 per patient to the overall costs, while chromogenic testing would have saved €29.88 per patient.

Conclusion: Chromogenic screening, but not PCR-based screening, can be considered a cost saving procedure to reduce unnecessary isolation days in patients at high risk for MRSA colonisation.

Evaluation of the impact of screening and signalling carriers of methicillin-resistant \textit{Staphylococcus aureus} on hand hygiene compliance and MRSA cross-transmission in 4 intensive care units

H. Hitoto, A. Koutchat, L. Dubé, A. Mercat, M.L. Joly-Guillou, M. Eveillard *, A.de Wit, B. van der Zanden, M. Bonten (Utrecht, Bilthoven, NL)

Objectives: To determine whether rapid diagnostic testing of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is cost saving when used for screening of pre-emptively isolated patients.

Methods: Incremental direct costs caused by addition of PCR (BD GeneOhm™ MRSA assay) or chromogenic agar (MRSA-ID by bioMérieux) to conventional cultures were calculated applying a hospital perspective. This included costs attributed to the rapid screening tests, costs because of false negative test results and savings because of avoided isolation days. The number of pre-emptive isolation days avoided with rapid diagnostic testing was determined in a prospective multi-centre experimental cohort study in 12 Dutch hospitals. This study included 853 patients.

Results: Costs of an isolation day, including supply costs and the extra time needed by nurses, physicians and cleaning personnel were estimated to be €27.30. The number of isolation days was reduced by 60.4% with PCR-based screening and would have been reduced with 47.4% with chromogenic agar screening. Cost per test, when added to standard culture procedures, was €52.70 for the PCR and €2.05 for chromogenic agar. Four false negative test results during the study resulted in additional costs of €10,077.41. The costs per isolation day avoided were €92.25 and €8.28 for PCR and chromogenic testing, respectively. Performing MRSA PCR added €143.73 per patient to the overall costs, while chromogenic testing would have saved €29.88 per patient.

Conclusion: Chromogenic screening, but not PCR-based screening, can be considered a cost saving procedure to reduce unnecessary isolation days in patients at high risk for MRSA colonisation.
money and time consuming. Our objective was to assess the impact of screening and signalling MRSA carriers on hand hygiene compliance (HHC) and MRSA cross-transmission in intensive care units (ICU).

**Methods:** We conducted an evaluative study with two 6-month periods in 4 ICU. During the study, a systematic screening was performed on patient admission and then weekly within hospitalisation. Screening results were not transmitted to clinical staffs in the first period (P1) but were transmitted and carriers signalled during the second period (P2). During P1, 200 contacts were observed without any awareness about the carriage status of patients. During P2, the first 200 contacts were observed independently of the carriage status of the patient, whereas the last 200 contacts observed were chosen in order to have the same number of observations for MRSA carriers and non-carriers during P2. The first comparison (C1) of HHC represented our main objective and concerned the contacts with MRSA carriers and the contacts with non-carriers in P2. The second comparison (C2) of HHC was performed to evaluate the overall impact of screening and signalling and concerned observations in P1 and the first 200 observations in P2. Lastly, a comparison of MRSA cross-transmission (MRSACT) (C3) was performed between P1 and P2. Two indicators were used: the incidence of acquired MRSA/1000 patient-days (I1) and the incidence of acquired MRSA/1000 days of hospitalisation of patients admitted with an MRSA.

**Results:** All categories of personnel were observed but 80% of the observations concerned nurses and nurse assistants. Overall, HHC was 43.5% (39.5% before contact vs. 43.1% after contact, P = 0.004). Concerning C1, the HHC for contacts with MRSA carriers was 42.5% vs. 43.1% for contacts with non-carriers (not significant). Concerning C2, the HHIC in P1 was 44.8% vs. 48.5% in P2 (not significant). Concerning C3, the 2 indicators were discordant. I1 was 3 fold higher in P2 than in P1 (2.0 (C95% = (1.97–2.03)) vs. 0.70 (C95% = (0.68–0.72)). I2 was higher in P1 (16.0 (C95% = (14.0–18.0)) than in P2 (11.4 (C95% = (7.4–15.4))).

**Conclusion:** We failed to identify any advantage by using screening and signalling MRSA carriers in those 4 ICU.

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**P1894 Costs of nosocomial methicillin-resistant Staphylococcus aureus pneumonia**

E. Ott*, K. Graf, F. Schwab, C. Reichardt, I.F. Chaberny (Hanover, Berlin, DE)

**Background:** Numerous studies demonstrated that nosocomial infections with methicillin-resistant *Staphylococcus aureus* (MRSA) are attended by increased morbidity and mortality. Furthermore they present a high financial burden for hospitals and community.

**Objective:** The purpose of this study was to investigate attributable costs for nosocomial MRSA-pneumonia inside the German DRG-System.

**Methods:** We conducted a case-control study including all patients (pts.) with nosocomial pneumonia caused by MRSA and by meticillin-susceptible *S. aureus* (MSSA) admitted between January 2005 and December 2007. Pneumonia was defined using CDC criteria. Nosocomial cases with MRSA-pneumonia were matched to control patients with MSSA-pneumonia in a ratio 1:1 using following matching criteria: admission in the same year, minimum length of stay corresponding to time at risk of the case, Charlson Comorbidity-Index ≥1, occurrence of pneumonia at intensive care unit (ICU).

**Results:** Our analysis includes 82 patients (41 cases, 41 controls). The median overall costs for patients with nosocomial MRSA-pneumonia were significant higher than for control patients (60,684€ vs. 38,731€, p = 0.001). Furthermore we detected a significant difference in the median financial loss for cases and controls (11,704€ vs. 2,662€, p = 0.002). The attributable costs for MRSA-pneumonia were 17,282€ in median (p < 0.001).

The acquisition of MRSA-pneumonia on ICU (ME = 2.6; p < 0.001), ventilation >500 h (ME = 2.6; p < 0.001), liver disease (ME = 1.5; p = 0.021) and MRSA-pneumonia (ME = 1.8; p = 0.01) were predictive for increased costs in multivariate regression analysis.

**Conclusion:** This study pointed out that nosocomial MRSA-pneumonia is associated with high costs for healthcare systems compared with MSSA-Pneumonia. Appropriate infection control measures will be cost-effective and therefore essential.

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**P1895 Eradication of an epidemic methicillin-resistance Staphylococcus aureus from a geriatric university hospital. Evidence from a >10-year follow-up**


**Objectives:** Published studies of successful eradication of meticillin-resistant *S. aureus* (MRSA) in health care institutions are scarce. We report the successful eradication of MRSA after an epidemic in the year 1992 involving 37 individuals in the geriatric hospital of the University Hospital of Basel, Switzerland, an institution with 142 beds and about 50,000 patient days per year with less than one MRSA patient/year before 1992.

**Methods:** After detection of the outbreak, a multifaceted intervention was performed including contact isolation precautions, optimising of infection control activities with a focus on hand disinfection, screening of all individuals at risk and decolonisation of all MRSA carriers. Identified MRSA carriers were strictly kept on contact isolation or cohorted until successful decolonisation. Decolonisation of MRSA carriers was performed using mupirocin, chlorhexidine and systemic antibiotics if indicated. All isolates from the epidemic as well as all MRSA isolates ever cultured from a patient of our institutions between 1992–2005 were typed by multiple molecular typing methods, at least by pulsed-field gel electrophoresis and the majority by spa typing.

**Results:** After identification of MRSA in 7 patients, a MRSA screening of all patients and staff members in the geriatric hospital was performed. Overall, 32 patients and 5 staff members were found to be MRSA carriers. 21/32 patients (66%) and all 5 staff members were successfully decolonised. 7/32 patients (22%) died during the epidemic before decolonisation. 2/32 patients (6%), a couple, were discharged with persisting MRSA colonisation, and 2(6%) were lost to follow-up. Systematic screening of 142 patients and all staff members after the epidemic in 1995 and 1997 revealed no MRSA carriers. Since more than 10 years, the strain was not anymore identified, based on epidemiological surveillance and molecular typing of all MRSA strains from any specimen submitted to the microbiology laboratory.

**Conclusion:** This study provides strong evidence that long-term eradication of an MRSA epidemic in a hospital is feasible, and endemicity of MRSA after an outbreak can be avoided. The successful bundle approach for eradication of MRSA during an epidemic is expensive, but the long-term benefits likely outweigh the initial heavy use of resources.
and resulted in difficulties in trend analysis. Using web-based technology an on-line form was designed to overcome these pitfalls.

**Results:** Development of the on-line web-based form allows the entire RCEA process to be completed electronically. Initially the electronic surgery form is populated with personal identifiable information and then links to other hospital based systems to acquire information about ward movements. The users are then taken through a series of targeted questions to establish risk factors and focus of infection, identify lessons to be learnt or where non-compliances with policies have occurred. All of the questions either have drop down boxes with options, or are date and time fields to enable meaningful analysis and comparison of cases. On completion of the RCA, there are a series of verification processes, firstly by the user and then by the infection control team. If key questions have not been completed the user will be asked to return to these. An action plan must be developed to cover the key learning points, for all actions the person responsible is nominated and the action plan link sent to them. On completion a comment is placed on the system and once all actions relating to a specific RCA are complete the case is considered closed. All entries carried out on the on-line system are tracked with date, time and user allowing audit of the process.

**Conclusion:** The on-line tool is easy to use, resulting in a reduced time to completion and analysis of the findings. This has allowed us to analyse and interpret the data enabling the introduction of targeted and effective infection control measures.

### Surgical site infections

**P1897 Region-wide surveillance of surgical site infections after orthopaedic surgery in Crete, Greece**

M. Roumbelaki, E. Kritsotakis, A. Messaratiki, E. Bolikas, C. Tsioitas, A. Gikas* on behalf of the Cretan Coordination Center for Nosocomial Infection Control

**Objectives:** In this first attempt to implement an active surveillance system of surgical site infections (SSI) in a network of hospitals in Greece, our objective was to identify areas for improvement by comparing SSI rates with international benchmarks and by specifying main epidemiologic features of SSI after orthopedic surgery.

**Methods:** The US National Nosocomial Infections Surveillance (NNIS) system protocols were employed to prospectively collect data for patients who underwent orthopedic surgery during an 11-month period in 7 hospitals in the region of Crete in Greece. Operative procedures surveyed included open reduction of fracture (FX), knee prosthesis (KPRO), hip prosthesis (HPRO), and spinal fusion (FUSN). Comparisons of procedure-specific SSI rates with international data were performed by means of indirect standardisation after stratifying the rates by the NNIS risk index, and were reported in terms of standardised infection ratios. Risk factors for SSI were evaluated by multivariate logistic regression.

**Results:** A total of 68 SSIs were detected in 1478 operations (4.6 per 100 operations), of which 46% were detected post-discharge. Among the recorded SSI, 44% were superficial, 52% were deep and 4% were organ-space infections. Antibiotic prophylaxis was administered for 97% of the procedures classified as clean and for 77% of clean-contaminated procedures, for a median duration of 6 days. Procedure specific SSI rates and risk-adjusted comparisons with international data are presented in the Table. SSI rates were significantly higher for 3 operation categories compared with US data (FX, HPRO, KPRO), and for 2 operation categories compared with Spanish data (FX, KPRO) and UK data (FX, HPRO). Independent risk factors for SSI included: Charlson comorbidity index >1 (odds ratio [OR]=1.8, p=0.024), wound class not clean (OR=2.2, p=0.035), and prolonged duration of operation (OR=4.0, p<0.001). The mean postoperative hospital stay was significantly longer for patients who acquired a SSI than those without SSI (28.6 vs 10.6 days, p<0.001). SSI was not associated with mortality.

**Conclusion:** This study demonstrated the feasibility of implementing a standardised surveillance protocol of SSI after orthopedic surgery in our region, created awareness of the magnitude of the problem of SSI, and generated data useful for designing targeted infection control interventions.

**P1898 Incidence and determinants of surgical site infections after colorectal surgery**

Y.J.A.M. Hendriks*, R.M.P.H. Crolla, J.A.J.W. Kluytmans (Breda, NL)

**Objective:** Surgical site infections (SSI) are well known complications of colorectal surgery. We determined the incidence and determinants of SSI after colorectal surgery.

**Methods:** Prospective follow-up from November 2007 until December 2008 of all patients undergoing colorectal surgery at our hospital. Patients with dirty or infected procedures were excluded from the analysis. SSI were defined by the criteria of the Centers of Disease Control. Follow-up included post-discharge surveillance until 42 days after the initial operation. The following variables were included in the analysis: Age, sex, Body Mass Index (BMI), ASA score, removal of hair, the number of operations performed by the individual surgeons, lowest body temperature during surgery, duration of the surgical procedure, use of prophylactic antibiotics, and elective or acute procedure. Univariate and multivariate analyses were performed and statistical significance was accepted when p<0.05.

**Results:** 282 patients undergoing clean-contaminated or contaminated colorectal surgery were included. The mean patient age was 66.5 years and 40% were female. 64% had a diagnosis of cancer. SSI were found in 56 patients (20%). 6.4% had a superficial incisional SSI and 13.5% and 40% were female. 64% had a diagnosis of cancer. SSI were found in 56 patients (20%). 6.4% had a superficial incisional SSI and 13.5% had deep incisional SSI or organ/space infections. In univariate analysis the following variables were associated with the occurrence of SSI (p<0.1): Surgeons with a low amount of procedures, acute procedures, no removal of hair before surgery, a high BMI and a longer duration of the surgical procedure. After multivariate analysis the following variables were statistically significant: BMI (p=0.037) and duration of the surgical procedure (p=0.006).

**Conclusions:** The incidence of SSI in patients undergoing colorectal surgery is high. The two variables that were associated with the occurrence of SSI (BMI and duration of surgery) do not offer an easy target for preventive interventions.

**P1899 Consequences of surgical site infections after colorectal surgery**

Y.J.A.M. Hendriks*, R.M.P.H. Crolla, J.A.J.W. Kluytmans (Breda, NL)

**Objective:** Surgical site infections (SSI) were found in 20% of patients undergoing colorectal surgery in our hospital. We estimated the consequences of SSI by determining the length of hospital stay and mortality.

**Methods:** Patients (n=282) who had undergone colorectal surgery between November 2007 and December 2008 were included in the analysis. SSI were defined by the criteria of the Centers of Disease Control. The patients were followed for the development of SSI including post-discharge surveillance for 42 days after the initial operation. Mortality was followed for 6 months after the initial surgical procedure.

**Results:** Of the 282 patients, 56 suffered from SSI (20%). The average age for the patients was 66.5 years. The operations were elective in 98% of the cases and 2% were urgent. Patients with a SSI had a significant longer post-operative stay (Mean: 28.3 versus 8.9 days, p<0.001) and a longer post-operative stay on the Intensive Care Unit (Mean: 4.2
Surgical wound infection after median sternotomy: Risk factors for fungal surgical site infections in recipients

Methods: Comparison of the incidences and analyzed factors in 2005–2006 versus 2008. The mortality in infected patients was significantly higher (see figure, p < 0.001).

Conclusions: The present study confirms an incidence of SWI acceptably low, despite the increased mean age and the higher rate of patient with emergent-urgent surgery in the present series. Although the adherence to guidelines and recommendations for preventing SWI remains quite low, comparing to the previous study all the risk factor resulted slightly better controlled.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Incidence of sternal wound infection</td>
<td>2.65</td>
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<tr>
<td>Mean age (years)</td>
<td>69.9</td>
</tr>
<tr>
<td>Mean preoperative hospitalisation (days)</td>
<td>9.74</td>
</tr>
<tr>
<td>Coronary artery bypass graft/valve replacement (%)</td>
<td>58.6/74.9</td>
</tr>
<tr>
<td>Elective procedure (%)</td>
<td>44.9</td>
</tr>
<tr>
<td>Cardiopulmonary bypass (%)</td>
<td>90.9</td>
</tr>
<tr>
<td>Length of surgery &gt; 5 hours (%)</td>
<td>22.3</td>
</tr>
<tr>
<td>Prophylactic hyperglycaemia (%)</td>
<td>22.7</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
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<tr>
<td>Patients with cultures for staphylococcal nasal colonisation (%)</td>
<td>52.2</td>
</tr>
<tr>
<td>Staphylococcal nasal carrier (% of cultured patients)</td>
<td>9.6</td>
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<tr>
<td>Shaving of operative site (%)</td>
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</tr>
<tr>
<td>Shaving of operative site at day of surgery</td>
<td>46.6</td>
</tr>
<tr>
<td>Prophylaxis with 1st–2nd generation cephalosporins (%)</td>
<td>75.4</td>
</tr>
<tr>
<td>Prophylaxis with glycopeptides – multiple doses (%)</td>
<td>16.4</td>
</tr>
<tr>
<td>Prophylaxis with glycopeptides – single doses (%)</td>
<td>8.1</td>
</tr>
<tr>
<td>Prophylaxis with non-antistaphylococcal agents (%)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Conclusions: The present study confirms an incidence of SWI acceptably low, despite the increased mean age and the higher rate of patient with emergent-urgent surgery in the present series. Although the adherence to guidelines and recommendations for preventing SWI remains quite low, comparing to the previous study all the risk factor resulted slightly better controlled.

Surgical site infections

P1900 Surgical wound infection after median sternotomy: incidence and risk factor analysis. Results of a large multi-centre study in Italy

C. Santini* on behalf of GIS-INCARD (Gruppo Italiano per lo Studio delle Infezioni in Cardiochirurgia)

Objectives: Sternal wound infection(SWI) after cardiac surgery is associated to high mortality and costs. The adherence to the recommendations to prevent SWI is usually low, independently from their ranking. Despite the weak control of several modifiable risk factors, the use of antibiotic prophylaxis was only partially acceptable in the previous study. Aim of the present study was to verify the incidence of SWI and to re-evaluate the adherence to standard recommendations in a large series of patients.

Methods: Nineteen divisions of Cardiac Surgery participated in this observational study. From March to September 2008, all the patients undergoing median sternotomy were followed for one month to detect SWI, defined according to the CDC diagnostic criteria. In a single day every 2 months, all the inpatients with median sternotomy had recorded several variables regarding patients and operation characteristics, co-morbidity, pre- and post-operative risk factors, Staph. aureus nasal colonisation, timing and modality of debridement and perioperative prophylaxis. All the data were inserted in a data base and analysed by a central supervisor.

Results: Overall, 3381 patients underwent median sternotomy; out of them 3043 (90.0%) were evaluated. Eighty SWI were observed (2.62%). Clinical variables referred to 610 patients were analysed. In table 1 we report the characteristics of patients and operations, the incidence of several risk factors and the antibiotics used for perioperative prophylaxis in the present and in the previous study. Comparing to the previous study, almost all the modifiable risk factors were slightly better controlled: preoperative hyperglycaemia (−5%), more patients had investigated for staphylococcal nasal carriage (−8%), less patients had hair shaving (−4%) and more patients had hair removal soon before surgery (−17%).

P1901 Risk factors for fungal surgical site infections in recipients of ventricular-assist devices

A. Schweiger*, C. Rauf, M. Wilhelm (Zurich, CH)

Objectives: Ventricular-assist-devices (VAD) are mechanical support systems to maintain blood-circulation in patients with terminal cardiac failure. The aim of our study was to evaluate potential risk-factors for fungal infections in VAD-recipients during the period between 2004 and 2008.

Methods: Retrospective comparison of 4 patients (3 male) with candida surgical-site-infection (SSI) of the VAD (3 C. albicans, 1 C. parapsilosis) to 8 consecutive patients without VAD-support during the same time period without candida infection. Information from medical records was retrieved on patient history, number of body sites colonised with Candida sp., antibiotic treatment, days in ICU, and days on mechanical ventilation, dialysis, gastrointestinal complications and/or abdominal surgery and antibiotic treatment.

Results: Median age at time of VAD-implantation was 55 years (44–59) in cases, and 33 years (16–61 years; p = 0.089, Mann-Whitney) in controls. 2 involved mediastium (both C. albicans), the other 2 only soft tissue. Median time to development of SSI after admission was 38.5 days (range 19–223) and 17 days (range 2–195) after VAD-implantation. For detailed clinical information see table. Candida colonisation of 4 body sites prior to infection was found in 2 patients, compared to 1 in the control-group (p = 0.236, Chi-square). Mechanical ventilation for >14 days (3/4 in infection group, 3/8 in controls) was significantly associated with development of candidal infection (p < 0.05, univariate logistic regression). Development of mesenteric ischaemia showed a trend as risk-factor (p = 0.053). Exposure to broad-spectrum antibiotic treatment (Mean 706 days/1000 observation days in infection group vs. 463 days/1000 observation days in controls, P = 0.1763) was higher in the infection group. No single antibiotic was associated with an increased infection risk. Other analysed factors as mentioned above were statistically not significant.

Conclusion: The only significant predictor for candida SSI in patients with ventricular-assist devices was mechanical ventilation for >14 days. Other analysed factors were not significant, possibly due to low number of cases. The optimum strategy for prevention of candida SSI (e.g. rigorous evaluation of candidal colonisation, minimising antibiotic pressure, pre-emptive antifungal treatment in patients suffering of mesenteric ischaemia) in VAD-recipients should be investigated in prospective studies.
Post-discharge surveillance of surgical site infections after total hip and knee arthroplasty

**Objective:** The aim of the study was to investigate the surgical site infection (SSI) rate after total hip (THA) and total knee (TKA) arthroplasties using postdischarge surveillance.

**Methods:** From January to June 2007 a total of 553 patients were evaluated after THA (n=253) and TKA (n=300) in Tartu University Hospital. SSI was identified according to the National Nosocomial Infection Surveillance system (NNIS) criteria during hospital stay and for 12 months postdischarge using telephone calls, laboratory data and medical record review. Potential prophylaxis-, patient-, and procedure-related risk factors were also collected prospectively.

**Results:** A total of 8 SSIs were identified. SSI was recorded in 5 patients after THA (cumulative incidence rate 2.4%): 3 deep or organ/space and 2 superficial SSIs. After TKA 3 SSIs were identified (cumulative incidence rate 1.0%): 1 organ/space and 2 superficial SSIs. Most of the operations were performed in patients with the NNIS risk index category 0 or 1 (47.0% or 51.4%, respectively). The incidence of SSI in THA and TKA stratified according to the NNIS risk index was 2.06% and 1.57% (NNIS = 0), and 1.72% and 0.59% (NNIS = 1), respectively. The total response rate to the telephone questionnaire survey was 95.3% and 2 out of three SSIs detected by telephone questionnaire were superficial. All the other cases were detected on readmission. The median length of hospital stay after operation was 5 days (range 3–24 days). Time between operation and detection of SSI cases ranged from 14 to 356 days. 72.1% of patients received antibiotic prophylaxis within 30–60 min before incision.

**Conclusions:** Although our numbers are small, all SSIs were identified using postdischarge surveillance. In our centre the incidence of SSI in risk index category 0 is higher than the notified incidence in the Hospitals in Europe Link for Infection Control Through Surveillance or NNIS system which needs further investigation.

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### [P1903] Associations between operative site microbical counts and procedure classification in neurosurgery


**Objectives:** Correlation between operative site skin bacterial counts in neurosurgical procedures and the development of surgical site infections (SSI) has not been proven. We evaluated the association of bacterial Colony Forming Units(CFU), type of procedure and development of SSI in a prospective pilot study.

**Methods:** Skin swab cultures were obtained within 1 cm from the incision; pre-, post-preparation and preclosure samples were obtained. Bacterial counts were enumerated and the most prevalent organisms were recorded. Procedures were classified as clean, clean with foreign body, contaminated, contaminated and dirty.

**Results:** 83 procedures (42.1% clean, 21.7% clean-contaminated, 18% clean with foreign body, 14.5% contaminated and 4.8% dirty) in 70 patients(70% male) were evaluated prospectively. Trauma was the most common reason for surgery (31.8%). 93 sample sets were cultured. 88.2% prepreg, 28% postprep and 43.5% of the preclosure samples tested positive. Coagulase-negative staphylococci (CoNS) were the most frequently isolated organisms irrespectively of sampling time (62.4%, 18.3% and 29.3% for samples 1, 2 and 3 respectively) and independently of procedure classification. The median cfu count log for CoNS were 3 log (range 2–6.3), 2.6 (range 2–3.7) and 2.4 (range 2–4) respectively for each sampling. *P. acnes* was the second most frequent pathogen isolated. The median cfu count log for *P. acnes* were 3.9 log (range 2.2–6.6), 3.2 (range 2–3.8) and 4.3 (range 2–5.4) respectively for each sampling. There was a significant difference in the *P. acnes* counts isolated from head versus other sites. Procedure classification or prolonged surgery duration were not associated with microbial counts irrespectively of sampling time. SSI development was not associated with bacterial CFU at any sampling, therefore there was a trend for association with CoNS pre-preparation CFU.

**Conclusion:** CoNS was the most frequent bacterial pathogen cultured irrespectively of sampling. The pathogen cfu log did not significantly differ among the samples. *P. acnes* was significantly more isolated from head specimens. Procedure classification was not associated with microbial skin counts at any sampling but CoNS prepreparation CFU carried a trend for statistical association with SSI development in this pilot study.

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### [P1902] Reducing surgical site infection rates in cardiac surgery: results of 10-year infection control programme

**R. Finkelstein*, G. Rahino, T. Maschiach, Y. Bar-El, Z. Adler, Y. Kerzman, O. Cohen, S. Milo (Haifa, IL)**

**Background:** To report the results of an infection control program on surgical site infections (SSIs) complicating cardiac operations.

**Methods:** Prospective cohort study of patients undergoing cardiac operations. Interventions included prospective surveillance, povidone-iodine scrub showers, depilation before surgery, administration of preoperative antibiotic prophylaxis in the operating room and comprehensive postdischarge follow-up. Infections were evaluated using CDC's definitions. Logistic regression models were fitted to assess infection rates over time, adjusting for factors known to affect SSI rates (NNIS risk index category, type of operation, sex, age, emergency operation, administration of preoperative antibiotic prophylaxis, length of stay in hospital before surgery).

**Results:** 3,249 consecutive procedures were evaluated from January 1st, 1997 to December 31, 2006. Rates of deep incisional SSIs remained low, but unchanged over the study period (mean 1.8%). The rate of superficial incisional SSIs (SUP), all organ/space infections (OS), and mediastinitis (MED) during the first two years were 4.7%, 3.1%, and 2.2%, respectively and they decreased to 2.6%, 1.31%, and 0.19%, respectively by the end of 2002 (p = 0.04, 0.07, 0.02). The rate of SSIs due to methicillin-resistant *S. aureus* (MRSA) decreased from 1.48% to
Evaluation of three preoperative preparation products when used in a preoperative site-wash regimen

C. Beasolí* (Bozeman, US)

Objective: Many patients undergoing orthopedic, cardiovascular, and general thoracic or abdominal surgeries experience post-surgical infections, prolonging their medical treatments, and imposing unnecessary medical risk and expense. The standard of practice for preoperative preparations (PreOp Prep) is to treat the intended surgical site with an effective topical antimicrobial immediately prior to a surgery, usually with prophylactic antibiotic therapy before and after the surgery. However, some medical practitioners currently prescribe for their presurgical patients, in addition, a preoperative site-wash (PreOp SW) regimen with the intention of reducing microbial populations residing on the skin prior to the routine site preparation at the time of the surgery. The logical rationale has been that such a combination procedure would reduce potentially contaminative microbial populations to levels far lower than could the PreOp Prep alone.

The purpose of this study was to evaluate effectiveness of a PreOp SW procedure by measuring reductions of normal and transient populations. Three different commercially available products were tested – TRISEPTIN® Water-Aided, MaxiClns®, and Chloraprep®.

Methods: Two products were evaluated on each human subject to provide 10 data files per product (15 subjects, total). Technicians applied the products per use-instructions to the skin of subjects’ knees once per day for 4 days of consecutive days. Microbial populations were sampled each day prior to and immediately following treatment. Performance of a product was evaluated in terms of its ability to reduce microbial populations progressively and total over the 4-day period of testing.

Results: All products tested produced significant reductions in the populations of microbial flora on the skin of the knee (see table).

<table>
<thead>
<tr>
<th>Sample time</th>
<th>Chloraprep®</th>
<th>TRISEPTIN®</th>
<th>MaxiClns®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Day 1</td>
<td>2.56 (0.60)</td>
<td>2.56 (0.60)</td>
<td>2.56 (0.60)</td>
</tr>
<tr>
<td>Post-Day 1</td>
<td>0.60 (0.37)</td>
<td>0.60 (0.37)</td>
<td>0.60 (0.37)</td>
</tr>
<tr>
<td>Pre-Day 2</td>
<td>2.08 (0.50)</td>
<td>2.08 (0.50)</td>
<td>2.08 (0.50)</td>
</tr>
<tr>
<td>Post-Day 2</td>
<td>0.58 (0.35)</td>
<td>0.58 (0.35)</td>
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</tbody>
</table>

Efficacy of chlorhexidine glucconate against Staphylococcus aureus in a respiratory epithelial infection model


Background: Staphylococcus aureus, a significant pathogen, can invade via skin or mucosal epithelium. Chlorhexidine glucconate (CHG) is a common antiseptic agent used at concentrations from 0.12% (oral rinses) to 4% (surgical site preparations) to minimise the risk of nosocomial infections. This study evaluated the efficacy of CHG against a clinical isolate of Staphylococcus aureus, MN8, using two in vitro respiratory epithelial infection models.

Methods: S. aureus MN8 invasion of lung epithelial cells (A549) was characterised using fluorescently labeled bacteria (BacLight, Molecular Probes), and flow cytometry. Extracellular fluorescence (bound but not internalised bacteria) was quenched using trypan blue. Confluent nasal (RPMI 2650) and lung (A549) epithelial cells were infected with S. aureus MN8 1×10⁵ CFU/mL for 15 min prior to treatment with CHG (2.0–128 µg/mL; 0.0002 to 0.0128%) for 2 h. Antiseptic efficacy was evaluated by serial diluting and plating supernatants. Intracellular bacteria were enumerated by washing the adherent cells with PBS, resuspending in Triton X-100, serial dilution and plating. Cytotoxicity was evaluated by Cell Titer Aqueous One (Promega).

Results: S. aureus was bound to A549 within 10 min (16% of fluorescence) with approximately 50% being internal; therefore, we determined the ability of CHG to kill intra and extra-cellular S. aureus. At 2 h, complete S. aureus killing was observed in the supernatant at CHG > 64 µg/mL; and cell associated was achieved at CHG > 128 µg/mL.

Economic aspects of deep sternal wound infections

K. Graf*, E. Ott, F. Helm, F. Schwab, A. Hucerich, I.F. Chaberny (Hanover, Berlin, DE)

Objective: Surgical site infections (SSI) are a very expensive complication in cardiac surgery. The total costs for coronary artery bypass grafting (CABG) surgery followed by deep sternal wound infection (DSWI) are estimated to be much higher than for normal, caused by expanded length of stay, reoperations and special treatment such as vacuum-assisted closure therapy and expensive antibiotic therapy. This study compares the length of stay and the total costs of patients undergoing CABG and developing a DSWI or not.

Methods: A case-control study was performed. The total costs were analyzed and compared to patients undergoing CABG without DSWI. Inclusion criterion for cases was the development of a deep sternal wound infection according to CDC-criteria during the hospital stay with CABG. Cases with a readmission were excluded. Controls were matched by having the same type of surgery (DRG), age±five years, gender, the same duration of preoperative stay±two days. The time until the development of the infection was taken as the minimum length of stay for the controls. Controls showing signs of infection during the hospital stay were excluded.

Results: 17 CABG patients with DSWI, between January 2006 and December 2007, were included in the study. The cases were matched to 34 controls. The average cost of CABG procedure plus treatment of DSWI was 34755.63 Euro per patient and almost two times higher than in controls.
Diagnosis of viral infections

**P1909** The combination of antibody bridging and avidity index methodology for reliable and simple determination of infection phase


Objective: Measurement of serum antibodies is widely used for diagnosing the state of viral infections. In order to improve the accuracy and simplicity of serodiagnostic testing, and to speed up the diagnosis, innovative approaches are needed. We propose a new methodology for serological assessment of the infection phase.

Methods: Our approach combines the antibody bridging assay scheme with the avidity index technique. Accordingly, serum antibodies bind with one arm to an antigen on the solid phase and with the other arm to a labeled antigen in solution. When the serum diluent is supplemented with a chaotropic agent, the formation of low-avidity immunocomplexes is prevented.

The aim of this study was to evaluate the suitability of the antibody bridging avidity index (ABAII) methodology for rapid in vitro serodiagnostic testing. We developed ABAII methods for two different fluorescence immunoassay platforms, the heterogeneous time-resolved fluoroimmunoassay (TR-FLA) and the separation-free (homogeneous) two-photon excitation (TPX) assay technique. The new methods for adenovirus specific antibodies were compared to the conventional class-specific detection and to the validated IgG-avidity measurement principle (N = 35).

Results: The results show that the TR-FLA methodology allows reliable assessment of IgG avidity with considerably simpler assay protocols than the conventional IgG-avidity tests. The measurement of antibody avidity eliminates the need for paired serum sampling.

Conclusions: The new methodology allows simpler and quicker serodiagnostic testing than possible before. Thus, the novel approach could provide a competitive and cost-efficient alternative for the conventional methods; especially when using the one-step TPX assay technique which enables high sensitivity analyses from a few microliter reaction volumes.

**P1910** Evaluation of the performance of RSV Respi-Strip® in comparison with cell culture and reverse transcriptase PCR

I. Wybo*, D. Piéard, D. Stevens, O. Soetens, S. Lauwers (Brussels, BE)

Objectives: Respiratory syncytial virus (RSV) infection is a common respiratory infection in young children, occurring as a winter epidemic in temperate climates. A rapid and reliable diagnosis is very important for prompt institution of infection control measures. The purpose of this study was to compare RSV Respi-Strip® (Coris-Biocenter), an immunochromatographic assay, with “in house” reverse transcriptase (RT) PCR and viral culture. The need for incubation of the sample in extraction buffer before insertion of the strip was also evaluated.

Methods: Hundred nasopharyngeal aspiration samples were submitted by the emergency department or the paediatric wards from October 15 to November 21, 2008. For viral isolation 3 cell lines were inoculated to optimise recovery: MRC5, Hep 2 and LLC-MK2. A multiplex RT-PCR followed by hybridisation was performed for the detection of RSV and 8 other respiratory RNA-viruses. RSV Respi-Strip kit® was used according to instructions from the manufacturer. Briefly, 0.25 ml of nasopharyngeal aspirate was mixed with 0.25 ml of extraction buffer and incubated at room temperature for 0, 1 and 10 min. Subsequently strips were inserted into the buffer suspension and incubated for 15 min prior to reading. To calculate test performance samples were considered as true positive if positive by cell culture or if positive by both PCR and RSV Respi-Strip® in culture negative samples.

Results: 42% of samples were positive by RSV Respi-Strip®. No discrepancies were detected between results obtained with the different incubation times of the sample in extraction buffer. 25% were positive by cell culture and 54% were positive by PCR. 45% were considered as true positive – 25 positive by cell culture and 20 culture negative but positive by both PCR and RSV Respi-Strip®. 55% were RSV negative. Sensitivity and specificity of culture, PCR and RSV Respi-Strip® were respectively 25/45 (56%) and 55/55 (100%); 45/45 (100%) and 46/55 (84%); 41/45 (91%) and 54/55 (98%).

Conclusion: With a sensitivity of 91% and a specificity of 98% this rapid test method can be relied on to make infection control decisions during RSV epidemics. By omitting the incubation step in extraction buffer, the assay time can be reduced from 25 to 15 minutes.
**P1911** Evaluation of the Copan flocked swab with UTM-RT medium for antigen detection of HSV, direct immunofluorescence of RSV and viral culture of HSV

_S. Viggen, S. Nys*, R. Cartauxels, K. Magerman_ (Hasselt, BE)

**Objectives:** Successful preservation of viral viability and antigens depends on specimen collection, transport and preservation system used. In this study, the newly developed nylon-flocked swab with universal transport medium (UTM), specially designed to optimise specimen collection and to minimise entrapment of the microorganisms, was compared with the Copan virus transport system for antigen detection of HSV-1, direct immunofluorescence of RSV and viral culture of HSV-1.

**Methods:** Viral culture of a PCR-confirmed HSV-1 positive clinical sample was harvested and serially diluted in MEM 2% culture medium (CAMBREX).

The Copan flocked swab with UTM-RT medium (UTM, Copan, 395C) and the virus transport system (VTS, Copan 147C) were inoculated in 100 mL of each dilution and preserved respectively at room temperature or 4°C for 2 hours. Each swab was inoculated on the cell lines A459, MRC-5 and Hep2 and controlled daily for the cytopathogenic effect of HSV-1. The serial dilutions of the positive clinical sample were used as a positive control.

For the antigen detection and direct immunofluorescence, 500 mL of the UTM cell suspension was pelleted, washed and resuspended in PBS. Twenty microliters of this final suspension was stained using standard procedures.

**Results:** The MRC-5 and A549 cell lines inoculated with samples of the UTM swab showed a cytopathogenic effect as soon as the positive control wells. Also, dilutions of UTM were sooner positive as compared to VTS. These differences were less clear in the Hep-2 cells.

There were no significant differences in results of HSV-1 antigen detection and direct immunofluorescence of RSV with UTM compared to VTS. However, the yield with UTM was higher as compared to VTS, even at low viral loads.

**Conclusion:** The flocked swab with UTM-RT medium showed an overall better recovery of HSV-1 after culture. Furthermore, the flocked swab seems to have a greater diagnostic sensitivity in the HSV-1 antigen detection and RSV direct immunofluorescence.

**P1912** Standardisation of cytomegalovirus antigenaemia assay by in vitro generation of peripheral blood leukocytes positive controls and comparison of two commercial monoclonal antibody pools and immunostaining techniques

_T. Baptista-Fernandes, A. Dias*, T. Marques_ (Carinaxide, PT)

**Objectives:** HCMV is responsible for high morbidity and mortality rate in immunocompromised patients. The HCMV antigenaemia test, which detects phosphoprotein pp 65 (ppUL83) in nuclei of peripheral blood leukocytes (PBL), is a rapid and sensitive assay that allows the detection of infection prior to the onset of clinical disease, and is useful for monitoring response to therapy. We present a model for in vitro preparation of HCMV positive controls and results from comparing two monoclonal antibodies for HCMV antigenaemia testing: MABs C10=C11 (Biotech) and MABs 1C3=AYM-1 (Argene). We also compared detection by immunofluorescence assay (IFA) and immunoperoxidase assay (IPA).

**Methods:** For positive controls, PBL from healthy donors were isolated and co-cultivated with wild strains HCMV infected MRC5. PBL recovered after infection, were fixed and stained with specific MABs, followed by IFA and IPA. For leukocyte extraction, we followed the protocol described by Gerna et al. 2×10^9 PBL were applied per slide, fixed in formaldehyde solution, permeabilised with nonidet (NP-40 solution), and immunostained by IFA, as well as IPA, with MABs against HCMV pp65 (Biotech and Argene).

**Results:** Our results showed that fibroblasts infected with wild strains, isolated from newborn urines with congenital infection, could efficiently induce pp65 uptake by PBL. A total of 1292 blood specimens from transplant recipients and human HIV+ patients were tested. 308 specimens were positive for HCMV with one or both MABs. We found a statistical significant difference between two pools of MABs. Argene pool showed a significant higher sensitivity (p=0.008355 Chi-Squared test) with 133 (86%) positive tests, than Biotest pool with 107 (69%) positive tests. On applying Wilcoxon signed rank analysis to the positive cell counts, Argene pool showed significant higher levels of positivity, and capacity of detecting low levels of viraemia (p=0.0001). Concerning immunostaining technique, results with IFA showed a lower rate of artifacts, a shorter processing time and a higher sensitivity.

**Conclusion:** The availability of a proper positive control can lead to development of standardisation protocols and quality programs. Data suggests that a method with two slides per patient (PBL 2×10^5 per slide), combined with formaldehyde fixation and NP-40 permeabilisation, Argene pool (MAB 1C3=AYM-1) and IFA immunostaining provides optimal results, with a considerable improvement of antigenemia HCMV assay.

**P1913** Analytical performance of the new Access CMV IgG assay

_N. Grozeau, B. Rousseau, V Potelle, M. Bullenger, J. Piovane, R. Falcou-Briatte, F. Boumiot*, R. Theis, O. Flecheux_ (Marnes-la-coquette, FR; Nyon, CH; Chaska, US)

**Objectives:** An automated CMV IgG assay has been developed by Beckman Coulter. The purpose of this study was to evaluate the analytical sensitivity: limit of Blank (LoB), limit of detection (LoD), limit of quantification (LoQ), as well as linearity, accuracy, imprecision and analytical specificity of the Access® CMV IgG assay.

**Methods:** LoB and LoD were determined using five negative samples and five unique samples below the assay cut-off, respectively: 20 replicates of each sample. LoB and LoD calculation were performed according to CLSI EP17-A procedure. The LoQ was determined with two samples tested in 40 replicates. Linearity was evaluated by testing high positive samples diluted from 3/4 to 1/16. The actual v. expected concentrations, as determined on Access and UniCel Dxi 800 systems, were analyzed by linear regression. Imprecision studies used negative, low, medium and high positive samples and included intra-assay, inter-assay and inter-lot determinations. Analytical specificity was determined by testing 122 samples obtained from patients with specific disease conditions.

**Results:** The Access CMV IgG assay displays LoB, LoD and LoQ of 1.9 AU/mL, 3.2 AU/mL and 7.0 AU/mL, respectively. The linear regression slope for linearity was 1.09 with a total mean recovery percentage of 97.9%. The mean percentage of recovery for accuracy was equal to 100% on the Access system and 101% on the UniCel Dxi 800 system. Imprecision studies demonstrated %CVs below 11% and 14% for intra-assay and inter-assay, respectively, independent of the system in use. Inter-lot studies exhibited a %CV lower than 10%. Among the 122 samples tested for the analytical specificity, 121 were found negative with the Access CMV IgG assay.

**Conclusion:** The Access CMV IgG assay provides excellent analytical performance, with the advantages of a rapid, automated, random-access immunoassay system.

**P1914** Comparative studies of the Access CMV IgG assay

_N. Grozeau, B. Rousseau, V Potelle, M. Bullenger, J. Piovane, R. Falcou-Briatte, F. Boumiot*, R. Theis, O. Flecheux_ (Marnes-la-coquette, FR; Nyon, CH; Chaska, US)

**Objectives:** A new quantitative Access CMV IgG assay, developed by Beckman Coulter for use on the family of Access® immunoassay systems, was evaluated to determine concordance (total, negative and positive agreement) with the AxSYM CMV IgG assay.

**Methods:** The concordance studies were performed with samples from negative blood donors (n = 400), non-selected hospitalised patients (n = 1,212), non-selected pregnant women (n = 622), transplant patient serum samples (n = 143) and HIV positive serum samples (n = 42). The comparative device used for these studies was the Abbott AxSYM
CMV IgG assay and a percentage agreement between both methods was calculated. Discrepant results were further tested with the bioMérieux VIDAS** CMV IgG assay.

**Results:**
- Blood donors: The agreement between the Access CMV IgG and the AxSYM CMV IgG assays was 99.75% (397/398). Two samples with equivocal results were excluded from the calculation. The discrepant sample was negative with the VIDAS assay.
- Non-selected hospitalised patients: The agreement between both assays was 98.76% (1194/1200). Three samples with equivocal results were excluded from the calculation. The Access CMV IgG assay displayed a negative and positive agreement of 98.69% (601/609) and 98.83% (593/600), respectively. Among the 15 discrepant results, 9 Access CMV IgG assay results (5 positive and 4 negative) were confirmed with the VIDAS CMV IgG assay.
- Pregnant women: The agreement between both assays was 99.36% (617/621). One sample with equivocal result was excluded from the calculation. Among the four discrepant results, three Access CMV IgG assay results (negative results) were confirmed with the VIDAS CMV IgG assay.
- Transplant patients: The agreement between both assays was 99.30% (141/142). One sample with an equivocal result was excluded from the calculation. The VIDAS assay result for the discrepant sample confirmed the AxSYM assay result.
- HIV patients: The agreement was 100% (42/42).

**Conclusion:** The Access CMV IgG assay provides good agreement with the AxSYM CMV IgG assay. The Access CMV IgG assay displays excellent results as confirmed with the VIDAS CMV IgG assay. The Access CMV IgG assay can be used on the Access systems, the high-throughput UniCel® DxI 800 Immunoassay System and the integrated UniCel DxCi systems.

**MRSA on a closed psychiatric ward**

W. Ebner*, J. Schlachetzki, M. Dettenkofer, J. Langosch (Freiburg, DE)

**Objectives:** Surveillance data regarding prevalence and spread of MRSA among psychiatric patients are missing. Psychiatric patients do not belong to the groups at risk for an infection with MRSA. Nevertheless psychiatric patients may transmit MRSA into sensible medical areas. Setting We report the case of an 85-year-old female index patient suffering from alcohol and benzodiazepine dependency and senile dementia, who was admitted on our closed ward (14 patients) in the psychiatric university hospital of Freiburg, Germany. She was screened for MRSA because her husband has been known to be a carrier of MRSA. She was found to be MRSA-positive in the swabs of the nostrils (further swabs not taken). Single room isolation was not possible. Instructions of the patient to personal hygiene or adequate hand disinfection or the body washing with chlorhexidine or octenidine were not possible. Mupirocin nasal ointment was applied according to the standard common. Infection control measures were limited and focused on regular hand disinfection of the medical staff.

**Methods:** When the carrier state of the index patient was identified the 13 other patients of the ward were screened by taking swabs from nose and throat (day 0). Swabs were taken in the same manner on day 3 and day 7 from 11 patients (two patients had been meanwhile shifted to an open ward and control swabs were not taken). Additionally, swabs from the nose of the two ward physicians were taken on day 7.

**Results:** None of the 13 patients screened were found to be MRSA-positive on day 3 and neither on day 3 and 7. In addition, the swabs of the two physicians were tested negative. The nasal swabs of the index patient were negative on day 3 after completion of mupirocin treatment. The observed compliance of the personnel to hand hygiene was high.

**Conclusion:** Extended hygienic measures when handling with MRSA-positive patients are usually not practicable on closed psychiatric wards. Some measures like the single room isolation are generally not desirable within the scope of psychiatric therapy. Our case report suggests that transmission of MRSA can be averted by the strict observance of standard hygienic measures, above all thorough regular hand disinfection after physical contact with MRSA-patients. A direct transmission of MRSA from patient to patient was not observed in our case. Reliable, systematic data regarding prevalence and spread of MRSA on psychiatric wards are still lacking.

**Diagnosis of CMV infection by serology: comparison of methods**

K. Tsiweriotsi, J. Meletiadis*, N. Siafakas, S. Damianidou, M. Bobola, L. Zerva (Athens, GR)

**Introduction:** Accurate diagnosis of CMV infection is important, but false positive IgM results occur even with established commercial assays. The frequent observation of IgM-CMV positivity during our routine testing with AxSYM (Abbott) necessitated the implementation of parallel testing with another IgM assay as well as IgG avidity testing.

**Material and Methods:** In our setting (750-bed tertiary health care centre) and during a 5-month period all serum samples submitted to the laboratory for IgG and IgM CMV testing were examined by AxSYM. Samples with IgM-positive or indeterminate results were additionally tested for IgM antibodies and IgG avidity by VIDAS (BioMérieux); obtained results were compared.

**Results:** Among 449 samples tested by IgM-AxSYM, 374 (83.3%) were negative, 58 (12.9%) positive and 18 (4%) indeterminate. All 18 IgM-AxSYM indeterminate samples were IgM-VIDAS negative (11 of high, 7 of moderate avidity). Out of 58 IgM-AxSYM positive samples, 8 were IgM-VIDAS positive (6 of high, 2 of moderate avidity); 6 indeterminate (4 of high, 2 of moderate avidity) and 4 negative (27 of high, 16 of moderate, 1 of low avidity). Overall, among the 76 patients with positive or indeterminate IgM-AxSYM results, high IgG-avidity was detected in 48 (63.2%), moderate in 27 (35.5%) and low in 1 (1.3%) patient. Concordant IgM positive or indeterminate results between AxSYM and VIDAS were obtained only for 14 samples (18.4%) including 10 of high and 4 of moderate avidity.

Agreement of positive results between IgM-AxSYM (positive or indeterminate) and IgG avidity testing (medium or low) was detected for 28 samples (36.8%). Among 14 samples positive or indeterminate by IgM-VIDAS, 4 demonstrated medium and none low avidity (28.3% agreement). Conversely, out of 27 samples exhibiting medium avidity only 4 (14.8%) were positive or indeterminate by IgM-VIDAS; the single sample demonstrating low avidity was IgM-VIDAS negative.

**Conclusions:** These results demonstrate that the serological diagnosis of CMV infection is problematic even when using established assays. The frequent detection of high IgG-avidity in IgM-AxSYM positive samples and the high rates of CMV infection diagnosis by AxSYM in a low risk population, point to a specificity problem of the assay. Results obtained by IgM-VIDAS did not correlate with avidity testing. Finally, the clinical significance of medium IgG avidity results remains undefined, while more relevant cutoff values need to be set.

**Performance evaluation of the Access CMV IgG assay performed on the UniCel DXC 880i in a French hospital**

A. Decoster*, S. Delplancque, C. Bazin (Lomme, FR)

**Objective:** The aim of the present study was to evaluate the performance of Access CMV IgG assay performed on the UniCel DXC 880i (Beckman, USA) in routine conditions in our laboratory.

**Methods:** A study was performed during 6 weeks on 181 sera from the laboratory routine collected for cytomegalovirus IgG testing in 59 children (0–18 old, 30 women, 29 men) and 122 adults (18–93 old, 74 women, 48 men). We have evaluated the performance of the Access CMV IgG (Beckman, USA) in comparison with the LIASON CMV IgG (Diasorin, Saluggia, Italy). Complementary testing was performed in case of discrepancies using (BioMérieux, Marcy l’Etoile, France).

**Results:** Correlation coefficient between Access and LIASON CMV IgG assays was found at 92.1% and the concordance between the results of the two tests was 96.1% (IC 95%: 92.2–98.4). We obtained 0 equivocal result with Access CMV IgG assay but 2 equivocal results
with LIAISON CMV IgG assay (11%). We obtained 98 positive results (54%), 76 negative results (42%) and 7 discrepant (4%) results with Access CMV IgG assay. All the discrepant sera was tested in duplicate by Vidas system: 3 were equivocal, 1 negative, 3 positive. Relative sensitivity and specificity were calculated for the two tests: specificity Access was 96.2% and sensitivity Access was 98%; specificity LIAISON was 97.4% and sensitivity LIAISON was 97%. Reproducibility and repeatability were also evaluated for the Access CMV IgG assay: variation coefficient was between 4.6% and 10.5%.

The ergonomics of the automated system UniCel DXC 880i on which Access CMV IgG assay performed on UniCel DXC 880i could be easily integrated and used in a laboratory.

Conclusion: The results obtained during this study demonstrate that Access CMV IgG assay performed on UniCel DXC 880i could adequately discriminate between IgG positive and negative samples and that it is valuable for the diagnosis of CMV infection by testing specific IgG in primary tube. Adapted to high throughput routine testing, Access CMV IgG assay performed on UniCel DXC 880i could be easily integrated and used in a laboratory.

**Performance characteristics of an anti-Variella zoster glycoprotein IgG ELISA**


**Objective:** To evaluate the performance of the VaccZyme™ anti-Variella zoster glycoprotein IgG EIA by comparison to a reference time resolved fluorescence immunoassay (VZV TRFIA), and confirmation of the assay reproducibility and linearity.

**Background:** Sensitive laboratory tests are required to measure anti-VZV antibodies as an aid in determining response to VZV vaccination and establishing the immune status of pregnant women exposed to VZV infection. ELISAs utilising glycoprotein antigen have been shown to offer greater sensitivity and show better correlation to FAMA (fluorescent antibody-to-membrane antigen) “gold standard” method than traditional assays utilising purified virus particles.

**Method:** Anti-VZV glycoprotein antibodies were measured using the VaccZyme™ anti-VZV glycoprotein IgG EIA kit (The Binding Site, UK) and a VZV TRFIA reference immunoassay (HPA, UK). The panel of 336 sera included 139 antenatal patients and 79 post vaccination sera. Based on TRFIA cut-off guidelines 63% had antibody levels ≥150mIU/ml, 26% had antibody levels <100mIU/ml and 11% were within the range of 100–150mIU/ml. Intra-assay reproducibility was determined on nine samples (20 replicates) and inter-assay precision was tested on ten samples on six separate occasions. Linearity was examined using three high titre sera including the international standard code W1044 (NIBSC).

**Results:**

<table>
<thead>
<tr>
<th>Performance characteristic</th>
<th>Anti-VZV gp IgG</th>
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</thead>
<tbody>
<tr>
<td>Intra-assay reproducibility (%CV)</td>
<td>2.3–3.6</td>
</tr>
<tr>
<td>Inter-assay reproducibility (%CV)</td>
<td>1.5–9.7</td>
</tr>
<tr>
<td>Linearity R²</td>
<td>&gt;0.999</td>
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<tr>
<td>Linearity % recovery</td>
<td>92</td>
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<tr>
<td>Sensitivity relative to TRFIA (%)</td>
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<td>Specificity relative to TRFIA (%)</td>
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<tr>
<td>Agreement relative to TRFIA (%)</td>
<td>98.3</td>
</tr>
<tr>
<td>Correlation coefficient (R²)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Conclusion:** The VaccZyme™ anti-VZV glycoprotein IgG assay demonstrates excellent linearity and reproducibility as well as good agreement compared to the reference TRFIA. The VaccZyme™ assay is a useful addition to the available assays for detection of anti-VZV antibody following vaccination or infection with VZV. In addition, the assay is ideally suited to automation for high throughput screening.
EVALUATION OF THE NEW VERSANT HIV-1 RNA 1.0 ASSAY

Significance of QuantiFeron®-TB Gold test for patients infected with HIV-1

Results: Of 88 selected sera submitted for parvovirus serology, 56 were Biotrin IgG-positive, and 53 of these 56 (95%) were Focus IgG-positive; 32 sera were Biotrin IgG-negative, and 31 of these 32 (97%) were Focus IgG-negative. Similar findings were observed for the IgM comparison: 27 of 27 (100%) Biotrin IgM-positive sera were Focus IgM-positive, and 57 of 60 (95%) Biotrin IgM-negative sera were Focus IgM-negative. One specimen was Biotrin IgM-equivocal and Focus IgM-positive. Serore prevalence of parvovirus antibodies in healthy adults using the Focus kits was 59% (24/41) IgG-positive and 5% (2/41) IgM-positive. In cross-reactivity studies, 62% (15/24) of sera positive for IgG to other infectious agents were Focus Parvovirus IgG-positive, and 10% (2/20) of sera positive for IgM to other infectious agents were Focus Parvovirus IgM-positive; these proportions did not significantly differ from the comparable percentages observed for the healthy adult serum panel.

Conclusion: Focus Diagnostics’ new V9-based Parvovirus IgG and IgM ELISA kits performed similarly to Biotrin’s B19-based Parvovirus kits. Parvovirus IgG and IgM prevalence rates, as assessed using the Focus kits, did not significantly differ when comparing a healthy adult serum panel and a cross-reactivity serum panel.

Evaluation of the new versant HIV-1 RNA 1.0 Assay (kPCR) for quantification of human immunodeficiency virus type 1 RNA

A. Amendola*, P. Zaccaro, R. Sabatini, A. Bibbo, M. Blosi, E. Patella, A. Natale, G. Rossi, M.R. Capobianchi (Rome, IT)

Objective: To compare performance of the new Versant HIV-1 RNA 1.0 (kPCR) assay (Siemens Healthcare Diagnostics) for quantification of HIV-1 RNA in clinical samples from HIV-1-infected individuals with Versant HIV-1 RNA v3.0 (Siemens Healthcare Diagnostics) and COBASA Ampliprep/COBAS TaqMan HIV-1 CAP-TCM (Roche Diagnostics) procedures. Versant HIV-1 RNA 1.0 (kPCR) and COBASA Ampliprep/COBAS TaqMan HIV-1 assays are both based on RT real-time PCR technology; Versant HIV-1 RNA v3.0 is based on DNA technology and was considered as reference method.

Methods: The study was conducted on 256 retrospectively collected plasma samples from HIV-1 infected individuals attending the outpatient care facility of the “Lazzaro Spallanzani” Hospital in Rome. Quantitative results were compared with correlation, linear regression, Bland & Altman method, and k-statistic analyses of log10 transformed HIV-1 RNA copy numbers.

Results: Agreement between Versant HIV-1 RNA 1.0 (kPCR) and other assays was elevated (>0.940) and high correlation coefficients were measured: r=0.9662, p<0.0001 between Versant HIV-1 RNA 1.0 (kPCR) and Versant HIV-1 RNA v3.0 (bDNA); r=0.9597, p<0.0001 between Versant HIV-1 RNA 1.0 (kPCR) and COBAS Ampliprep/COBAS TaqMan HIV-1. Analysis of mean differences of measurement between assays, conducted according to Bland & Altman method, showed no clinically significant differences in quantification of viral load (lower than 0.2log10 cp/ml) along all the overlapping range.

Conclusion: Versant HIV-1 RNA 1.0 (kPCR) assay for quantification of HIV-1 RNA in plasma samples from HIV-infected individuals is based on RT real-time PCR technology. This new commercially available diagnostic system produces viral load results that can be considered equivalent to results given by the reference diagnostic system and a similar RT-real-time PCR-based procedure.

Significance of QuantiFeron®-TB Gold test for patients infected with HIV-1

M. Dimu*, L. Gavrilu, M. Iosipenco, C. Craciun, C. Baicu, D. Otelea (Bucharest, RO)

Objective: Viral load testing, antiretroviral treatment resistance and other immunological tests like CD4+/CD8+ counts are used to monitor patients infected with HIV-1. Previous studies showed that when HIV-1 viral load is higher CD4+ values are small, because lymphocytes TCD4+ are the most affected from HIV. Some studies revealed the capacity of HIV-1 to change the tropism in vivo and CD8+ lymphocytes could be transiently infected by HIV-1. Theoretically because of the decrease number of TCD4+ and TCD8+, QuantiFeron is not recommended when the immunological status of patients is affected. When the number of CD4+/CD8+ cells is low the level of IFN-γ released by lymphocyte stimulation, is too low to be meaningfully evaluated by ELISA. IFN-γ is a lymphokine, a subset of cytokines family, secreted by Th1, Tc, dendritic and NK cells. It has antiviral, immunoregulatory and anti-tumour properties.

HIV pandemic influenced the tuberculosis epidemiology in many countries. HIV is a risk factor for passage from latent tuberculosis to an active form of the disease. In HIV seropositive patients after infection with Mycobacterium tuberculosis, the number of TCD4+ decreases drastically for a short period and, as previous studies reported, the HIV-1 viral loads increase.

The aim of our study was to determine if the test is useful for those patients HIV-1 seropositive who are monitored on TARV.

Methods: We used for QuantiFeron testing a panel of 298 samples from HIV-1 positive patients; in addition viral load levels and TCD4+/TCD8+ counts were determined.

Statistical analysis used were Spearman Rank correlation, Student’s test and Mann-Whitney U.

Results and Conclusions: Correlation for QF-HIV VL: r=0.066, p=0.272; for QF-CD4+ count: r=-0.032, p=0.605; Mann-Whitney U association of QF-HIV VL (p=0.771) and QF-CD4 count (p=0.06) reveals no correlation between these results which indicates that the test can be used even for those patients who have low CD4 counts.

We wanted to compare the results of QuantiFeron test within the dynamical range of HIV viral loads and we noticed that the percent of indeterminate results increased proportionally with increases of viral load: for undetectable viral load we obtain 15% indeterminate results for QuantiFeron test, the second category (1 log–7 log RNA HIV-1 copies/ml) 17%, the third category (4 log–7 log RNA HIV-1 copies/ml) 28%. A possible explanation for these results is that virus changes the tropism for TCD8+ and affects all susceptible cells.

Figure 1. QuantiFeron test–HIV viral load association p=0.02 (QF: 1 = negative; 2 = positive, indeterminate was not considered).
Performance evaluation of VIDIA® HIV DUO, a new automated immunoassay test for qualitative HIV antigen/antibodies detection

V. Lemee*, R. Croise, F. Descamps, F. Barin, V. Barlet, M. Maniez-Montreuil, G. Kreplak, H. Menan, J.C. Plantier (Rouen, Metz-Tessy, Lille, Tours, Paris, FR; Abidjan, CI)

Objectives: We performed a study to evaluate the performances of the qualitative 4th generation combined antigen/antibodies VIDIA® HIV DUO (bioMérieux, France) test in terms of sensitivity and specificity.

Methods: The VIDIA HIV DUO assay principle combines a two-step enzyme immunoassay sandwich method with chemiluminescence detection. The raw materials selected enabled to detect HIV1 groups M and O, and HIV2 antibodies as well as Antigen from HIV1. Specificity was studied using 5160 fresh blood donor samples, 203 fresh blood hospitalised patients samples, 98 risk behaviour individuals samples and 124 potentially interfering samples. Specimens for diagnostic sensitivity evaluation included 608 characterised HIV positive samples and 17 primary HIV infection samples. For analytical sensitivity evaluation, 30 commercial seroconversion panels were included and P24 analytical sensitivity was established from range dilution according to the International reference reagent HIV-1 P24 antigen (NIBSC, United Kingdom) and French Society of Blood Transfusion Antigen Standard from BIORAD (Paris, France). The performance characteristics of this new product were established during the verification stage of the development of the product in various geographic sites.

Results: Specificity determined on blood donors was 99.88%, hospitalised patients samples and risk behaviour individuals was 100%, 1 sample syphilis positive was react with VIDIA HIV DUO. Diagnostic sensitivity was 100%. As regards the seroconversion and the performance panels, the results with VIDIA HIV DUO were comparable to those provided by the panel’s manufacturer and appear as sensitive as the majority of antigen tests for detection of primary HIV infection. HIV antigen sensitivity against the International reference, NIBSC HIV1 P24 antigen was evaluated to 0.5IU/mL. The analytical sensitivity against the BIORAD antigen was evaluated to 15.74 pg/mL.

Conclusion: This new HIV 4th generation assay shows excellent performance in terms of sensitivity and specificity. Results on the seroconversion panels are an added value for patients in terms of diagnosis and prevention. The combination of automation and high performance makes this product an excellent test for routinescreening.

Assessment of the Siemens ADVIA Centaur® HIV Combo assay for routine use in a virology laboratory

H.J. Fleury* (Bordeaux, FR)

Objective: To compare the performance of the new Siemens ADVIA Centaur HIV Combo assay (CHIV) with the Siemens ADVIA Centaur Enhanced HIV 1/2 (EHHV) and the Bio-Rad Genscreen™ ULTRA HIV Ag-Ab assays that are used in our virology laboratory for routine HIV testing. In addition, further comparisons were made using seroconversion panels and other commercially available known non-B HIV-1 samples.

Methods: Clinical samples were collected from hospitalised patients (1864), milk donors (7), individuals from an IST screening centre (212), pregnant women (99) and dialysed patients (61). Seroconversion panels samples (4), plasma samples with HIV-1 subtype B (60) and with non-B subtypes (38) coming from our sample bank were also tested. Both CHIV and EHHV assays were run on the ADVIA Centaur XP Automated Immunoassay System. Discrepant samples were centrifuged and duplicate tested with EHHV and Genscreen ULTRA HIV Ag-Ab assays and, when available, western blot and molecular data (RNA viral load). Seroconversion panels used were PRA801, PRB916, PRB944, PRB945 and PRD952. In addition, 29 control plasma samples with non-B HIV-1 subtype A, B, C, D, F, H, CRF01-AE and CRF02-AG were also tested during this study. A total of 2345 samples were tested.

Results: Based on seroconversion panels, the sensitivity of CHIV for detection of seroconversions was excellent with a positive result between 7 and 10 days after first bleed; the p24 antigen sensitivity of the assay was less than 125 HIV p24mIU/mL (WHO traceable via BBI panel) (10 pg/mL DuPont Standard, 38 pg/mL Sanofi Standard). After resolution of initial discrepancies, 2176 samples were found negative while 169 were positive and 8 still discrepant. The resolved sensitivity of the CHIV assay was 100% and the resolved specificity 99.64%. The CHIV assay allowed the detection of antibodies and/or p24 antigen in all B and non-B characterised samples.

Conclusion: Taking into account all data of this evaluation (sensitivity for detection of primary infections and p24 antigen, detection of B and non-B HIV infections, sensitivity and specificity evaluated on more than 2000 samples from a French university hospital) we consider that the Siemens ADVIA Centaur HIV Combo assay is suitable for routine use.

Comparability of results obtained with ELECSYS® Anti-HCV and Beckman Coulter HCV-ab using routine laboratory samples

P. Rieger* (Oberhausen, DE)

Objective: The objectives of the evaluation were to check the comparability of results obtained with Elecsys® Anti-HCV (on MODULAR Analytics E170) and Beckman Coulter HCV ab® (on DxC 880i) in the field using routine laboratory samples.

Methods: Left-over serum or plasma samples from the daily routine of a general hospital laboratory were first measured with Elecsys® Anti-HCV on MODULAR Analytics E170 and than with Beckman Coulter HCV ab® on DxC 880i. Discrepant results were verified with Abbott AxSYM Anti-HCV® and/or immunoblot.

Results: The performance of Elecsys® Anti-HCV was compared to the current Beckman Coulter HCV ab® on the DxC 880i analyzer in a clinical environment. The evaluation has been performed at a clinical laboratory in Oberhausen. The sample material were 603 anonymised left over serum/plasma samples from daily routine. The samples were measured with Elecsys® Anti-HCV and Beckman Coulter HCV ab®. Discrepant results were verified with Abbott AxSYM Anti-HCV® and/or immunoblot.

With a specificity of 99.66% compared to 99.16%, Elecsys® Anti-HCV showed a superior performance compared to Beckman Coulter HCV ab®.

Conclusion: In an experimental setting that represents the routine workload of a common hospital laboratory Elecsys® Anti-HCV on MODULAR Analytics E170 showed a superior specificity compared to Beckman Coulter HCV ab® on Beckman Coulter DxC 880i analyzer. With a specificity of 99.66% compared to 99.16% with Beckman Coulter HCV ab® Elecsys® Anti-HCV was better by 0.5%.

Evaluation of fully automated assays for the detection of anti-rubella IgM and IgG antibodies on the Elecsys® immunoassays system

J. van Helden*, L. Grangeot-Keros, K. Vlemmixs, F. Masset, M.G. Revello, E. Pfaffenrot, W van der Helm (Monchengladbach, DE; Paris, Coquettes, FR; Puissi, IT; Rothkren, CH)

Objectives: Screening for acute rubella infection in pregnancy is an important element of antenatal care due to the potential risk of birth defects associated with congenital rubella syndrome (CRS) arising from primary infection, particularly in the first trimester. Vaccination programmes have reduced the incidence of both acute rubella infection and CRS but coverage of the population is incomplete. This study compared the sensitivity, specificity and reproducibility of fully automated Elecsys® Rubella IgM and IgG immunoassays designed for the Elecsys 2010, Modular analytics E170, cobas e 411 and cobas e 601 analytical platforms, with current assays.

Methods: Elecsys Rubella IgG and Rubella IgM are electrochemiluminescence immunoassays which use recombinant rubella-like particles
and monomeric E1 antigen, as a validated alternative to authentic rubella virus. Comparisons with current methods were performed in clinical laboratories in France, Germany and Italy. Studies of assay sensitivity and specificity were done using frozen serum specimens. Fresh serum samples from routine pregnancy screening were also used for method comparison.

**Results:** Reproducibility of test results for the Elecsys Rubella IgM and IgG assays showed good between-run and within-run precision. The Elecsys Rubella IgM assay demonstrated a sensitivity of 80–96% in primary, early (<30 days) acute infection, similar to existing assays. The Elecsys Rubella IgG assay exhibited high seroconversion sensitivity in specimen samples after Rubella vaccination. The average time interval to the first positive bleed was 14.1 days with the Elecsys Rubella IgG assay and 19.7 days with the comparison assay. The Elecsys Rubella IgM assay revealed high specificity (98.7–99.0%) in fresh samples obtained from clinical routine antenatal screening (n = 1,556); and a statistically lower reactivity towards persistent Rubella IgM when compared to the reference assays. Resolved relative sensitivity of 99.9–100% and resolved relative specificity of 97.4–100% was found for the Elecsys Rubella IgG assay in pre-selected frozen samples and fresh samples from routine antenatal screening.

**Conclusion:** The Elecsys Rubella IgM and IgG assays provide convenient and reliable determination of anti-Rubella antibodies, with specificity, sensitivity and reproducibility that is comparable with assay systems in current use. Adoption of this new assay can help in the monitoring of rubella status in pregnant patients.

**P1928** A ten months’ results of a commercial enzyme immunoassay for detection of norovirus in outbreak specimens

H. Tuokko* (Oulu, FI)

**Objectives:** Norovirus is a major cause of nonbacterial gastroenteritis especially in hospitals and nursing homes. The aim of this work was retrospectively to study the ability of a commercial enzyme immunoassay to detect norovirus antigen in specimens taken in outbreaks and sporadic cases of gastroenteritis in the Northern part of Finland in 2008. A total of 1,064 diarrhoea specimens were collected for viral and bacterial tests in ten months from patients in hospitals, homes, health care centres and departments of Oulu University Hospital.

**Methods:** Norovirus was detected by RIDASCREEN Norovirus enzyme immunoassay (EIA) (R-Biopharm, Darmstadt, Germany). Noro RT-PCR (Huslab, Helsinki, Finland) was used to identify 22 specimens.

**Results:** Altogether 188 (17.7%) specimens were norovirus-positive. Concerning age groups of patients, the highest noropositivity was detected in specimens from children around one year of age (39.1%), the lowest 11.9% from patients with age of from 10 to 65 years, 20.3% positivity in specimens from patients older than 65 years. The major amount of the patients (732) were in age of from 75 to 95 years with noropositivity of 22.3% in their samples. In faecal samples of the seven largest outbreaks, taken during 2 to 3 weeks from the start, noropositivity was (60.6±15.5%), during 1 month (50±18%) and in the following months (40.1±8.5)%. Those 22 specimens included 14 positive and 8 negative NoroRT-PCR samples. The sensitivity of RIDASCREEN Norovirus EIA was 71.4% compared to NoroRT-PCR. The other findings were one Rota positive and 22 diarrhoea bacteria positive samples.

**Conclusion:** RIDASCREEN Norovirus EIA, rapid, easy to carry out and economic, detected as noro positive (60.6±15.5%) of the outbreak specimens taken during 2 to 3 weeks. Although the sensitivity of 71.4% to NoroRT-PCR, the kit, like this with monoclonal antibodies against different noroviruses, could be recommended (as screening test) for noro diagnosis in outbreak samples, but not for single-specimen in microbiological laboratories.

**P1929** Automated Enzymognost Rubella portfolio (Siemens Healthcare Diagnostics) as an important aid during 2008 rubella outbreak in Italy

A. Marangoni*, A. Moroni, S. Accardo, R. Cevenini (Bologna, IT)

**Objectives:** Rubella virus (RV) infection causes a benign disease in immunocompetent individuals, but may lead to congenital infection with serious sequelae for the newborn after primary infection in pregnant women. Accurate IgM, IgG and avidity assays are critical to diagnose RV infection and to clearly understand the serological status of patients. Although vaccination has substantially reduced the incidence of RV infection, Italy has faced an upsurge of RV cases since the end of 2007, with outbreaks reported in many regions. Here we report results obtained from September 2007 to August 2008 for sera submitted to the Microbiology Laboratory of S. Orsola Hospital, Bologna for RV serological testing.

**Methods:** Sera were tested by Enzymognost Rubella IgG and Enzymognost Rubella IgM, processed using BepIII analyzers (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). In both the kits the difference between OD values in the wells coated with antigen (Ag) and in the wells coated with control antigen (CoAg) is a measure of the concentration of RV antibodies detected in the samples. Sera showing OD > 0.200 values in CoAg wells of IgM assay were considered reactive and they were retested by VidasRub IgM (bioMerieux, Marcy l’Etoile, France), as well as all the samples scored positive or Borderline.
Finally, IgG avidity test was performed by using Urea 3.6M in combination with Enzygnost Rubella IgG. The antigen/antibody dissociating agent (Urea) was used in parallel with the usual IgG antibody assay, and the OD obtained with Urea was compared with the OD obtained without Urea, yielding an AI (Avidity Index), as follows: AI = OD with urea/OD without Urea.

**Results:** During the study period we screened 3409 serum samples for IgG and IgM anti-RV. IgM testing was repeated by Vidas Rub IgM, because of screening positive (127), Border-Line (61) or suspective results (112). Moreover, 245 samples were tested by IgG avidity test. Low avidity results were obtained in 59 samples, whereas 12 sera showed intermediate avidity: as expected, these 71 sera were scored positive by both IgM methods.

**Conclusion:** The IgG avidity test can be used to rapidly distinguish between acute RV infection and long lasting of IgM on the first sample from a patient. The implementation of the avidity test on the automated BEEPiL system has allowed us to perform this test in the daily routine. Moreover, in this study we confirmed the good performances of Enzygnost Rubella IgM as screening test.

Table 1. IgM results obtained by Vidas Rub IgM when sera scored positive, Border-Line or suspective by Enzygnost Rubella IgM were tested.

<table>
<thead>
<tr>
<th>ELISA</th>
<th>ELFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Positive (127 sera)</td>
<td>9 (7.1%)</td>
</tr>
<tr>
<td>Border-line (61 sera)</td>
<td>22 (36.0%)</td>
</tr>
<tr>
<td>Suspective (112 sera)</td>
<td>112 (100.0%)</td>
</tr>
</tbody>
</table>

It is worthy to underline that no suspective sera (i.e. showing OD larger than CoAg limit of IgM ELISA assay) were reactive by ELFA, confirming that all these sera had simply nonspecific reaction, correctly identified by screening test.

**P1930** Detection of antibodies to West Nile virus in people with schizophrenia using recombinant prM and E proteins produced by COS-1 cells


**Aim:** Antibodies to West Nile virus (WNV) have been found in many warm blooded animals and people and cause fever and encephalitis. As it is known, neurotropic viruses like WNV and Bornavirus disease virus with a predilection for latency are potential associates. However, the role of WNV infection in schizophrenia is not well documented. Therefore, we aimed to investigate the frequency of antibodies to WNV and its association with neurological diseases like schizophrenia.

**Method:** 200 sera from patients with schizophrenia were collected from Bakırköy Mental Health Hospital. 100 sera from healthy controls (HC) were collected from blood donors and 100 sera from the patients with other anxiety and depressive disorders were collected from the Psychiatry Department of the Cerrahpasa Medical Faculty. HC were blood donors with no history of non-progressive and non-infectious neurological disorders. The sera were analysed by ELISA using the recombinant antigens (prM and E) of WNV produced by COS-1 transformed cells. The home-made of ELISA showed the optimal concentration of antigen as 6 µg/ml and the optimal dilution of serum as 1:40. Human sera were therefore tested at this dilution. Each serum was tested in antigen coated, non-coated and COS-1 cell-coated wells to measure the background optical density. Sera that read at least 2-fold higher compared with background were considered positive.

**Results:** We detected higher WNV antibody titers only in one case (no: 128). Bornavirus IgG was also detected. No positivity was detected in any other patients and HC.

Patient (no:128): She is 70 years old and born in Adana city (Cukurova region of Turkey) which the endemic diseases transmitted with vectors (especially mosquito) are most commonly found. She was hospitalised in Bakırköy Mental Hospital for 40 years and still taking antipsychotic therapy. WNV antibody titer was highly elevated in this patient (Patient OD = 1.479, OD of control = 0.240).

**Conclusion:** In conclusion, the results of this preliminary study showed that antibodies to WNV in people do not seem to be frequently associated with schizophrenia and other psychiatric disorders. In spite of that, detecting high WNV antibody titres in one patient suggests us to consider the possibility of relationship between WNV infections and psychiatric diseases in endemic areas. However, a secondary test is needed. Therefore, we have extracted RNA from the blood of those patients and the presence of WNV will be analysed by real-time RT-PCR.

**Paediatric infections**

**P1931** Antibiotics versus placebo or watchful waiting for acute otitis media: a meta-analysis of randomised controlled trials

E. Vouloumanou*, D. Karageorgopoulou, M. Kazantzis, A. Kapaskelis, M. Falagas (Athens, GR)

**Objective:** Recommendations on withholding antibiotics in children with acute otitis media (AOM) have been inadequately implemented in clinical practice. We evaluated the role of prescribing antibiotics for AOM.

**Methods:** We searched PubMed and Cochrane. We performed a meta-analysis of randomised controlled trials (RCTs) that compared antibiotic treatment to placebo or watchful waiting (delayed antibiotic treatment if clinically indicated) for patients with AOM.

**Results:** We identified 7 trials comparing antibiotic treatment to placebo (all double-blinded), and 4 trials comparing antibiotic treatment to watchful waiting (2 investigator-blinded, 2 open-label) trials, all of which involved children (6 months to 12 years). Clinical success was more likely with antibiotics than comparator treatment, in placebo-controlled trials [7 RCTs, 1405 patients, odds ratio (OR)=2.00, 95% confidence interval (CI)=1.34–2.99]; watchful waiting trials (4 RCTs, 915 patients, OR=2.22, 95% CI=1.20–4.13); and all trials (11 RCTs, 2320, OR = 2.06, 95% CI=1.49–2.84). Similarly, persistence of symptoms 2–4 days after treatment initiation was less likely with antibiotics in placebo-controlled trials (4 RCTs, 1014 patients, OR = 0.57, 95% CI=0.43–0.76); and all trials (5 RCTs, 1299 patients, OR = 0.53, 95% CI=0.41–0.69). Diarrhoea was more likely with antibiotics (7 RCTs, 1807 patients, OR = 1.59, 95% CI=1.18–2.16). No differences between the compared treatments were found regarding other effectiveness and safety outcomes.

**Conclusion:** Antibiotic treatment is associated with a more favourable clinical course in children with AOM, compared to placebo, and to watchful waiting, as well. However, safety issues and the rather small treatment effect difference render the consideration of additional factors necessary in relevant clinical decision-making.

**P1932** Nasopharyngeal microbiota of children with acute otitis media (AOM) versus children with upper respiratory infection without AOM

L. Lindholm*, A. Ruohola, P.A. Tähtinen, M. Keinänen, R. Vainionpää, P. Huovinen, J. Jalava (Turku, FI)

**Objectives:** Nasopharyngeal microbiota, which consists of both bacteria and viruses, is a complex ecosystem which structure and function are inadequately characterised. Nasopharynx is the gateway to microbes to cause respiratory diseases. Thus, it is fundamental to know the microbial species and to elucidate the interactions between bacteria and respiratory viruses. The aim of this study is to compare the nasopharyngeal microbiota between children who have acute otitis media (AOM) and those who have uncomplicated upper respiratory infection (URI).

**Methods:** Nasopharyngeal specimens were taken from total of 557 children (aged 6–35 mo) in whom AOM was suspected by parents.
Nasopharyngeal bacteriota was analysed by semi-quantitative culture-based methods and respiratory viruses by PCR and antigen detection methods. In 319 children AOM was diagnosed and URI group consisted of 238 children with healthy ears. The nasopharyngeal microbiota of these two groups was compared.

Results: The composition of bacteriota and viruses in both groups (AOM vs. URI non-AOM) are shown in Figure 1.

Methods: The otitis media study followed children from 1 month to 5 years of age. Study was started in December 2006, data collected until June 2007 were included. *S. pneumoniae* strains (n = 61) and *H. influenzae* strains (n = 25) were isolated from 133 children. If AOM was diagnosed, myringotomy was performed to verify diagnosis of AOM and middle ear fluid was aspirated for culture of bacterial pathogens. Clinical specimens cultured in laboratory following standard procedures, susceptibility testing according CLSI guidelines. Pneumococci were serotyped using the Quellung method using specific antisera (Statens Serum Institut, Denmark). Paediatricians prescription habits in AOM were analyzed retrospectively from the General health insurance fund data.

Results: In 133 of patients, the tympanic membrane was perforated and ear discharge observed. In 61 of these cases (45.9%) *S. pneumoniae* and in 25 cases (18.8%) *H. influenzae* was cultured. The overall coverage of serotypes contained in the 7-valent conjugate vaccine causing otitis media in children under 5 years of age was 85.5%. High prevalence of nonsusceptibility to penicillin (51.8%), erytromycin (47.5%) and cotrimoxazole (75.4%) was observed. In our collection of pneumococcal strains serotype 23 F (29.5%) and 14 (24.6%) are leading serotypes. 20 strains (80%) of *H. influenzae* strains were nontypable, 1 strain was serotype 1, 5 strains serotype B and 1 strain serotype F. In total 2 strains (8%) were resistant to ampicillin. 75,846 patients insured in General Health Insurance Fund were treated by AOM in year 2007. 51% of them were children <5 year old. Total costs for antibiotic prescriptions for children <5 year were 139,727 €. In prescriptions were dominant cephalosporins (33.2%) and combinations of penicillins (31.9%).

Conclusions: The present study reports a 88% coverage of the 7-valent pneumococcal conjugate vaccine of pneumococcal AOM strains. Serotypes 23F and 14 were most common among *S. pneumoniae* AOM isolates. Prospective surveillance for AOM among children, vaccination, consumption of antibiotics and resistance is going on.

Figure 1. Bacterial and viral findings in the nasopharynx of patients with acute otitis media (AOM) vs patients with upper respiratory infection without AOM.

Respiratory virus was detected in 82% of the samples in both groups (AOM 81.5%, URI non-AOM 81.9%). Typical bacterial pathogens of AOM (*S. pneumoniae, H. influenzae, M. catarrhalis* and also *S. aureus*) were found in 309 (97%) AOM patients but only in 188 (79%) URI cases. Pathogenic bacteria and viruses were concomitantly found in 252 (79%) of AOM patients and in 153 (64%) of URI non-AOM patients. AOM patients had significantly (P = 0.0002) less non-pneumococcal streptococcal species in their nasopharynx than URI non-AOM patients.

Conclusion: Our comparative data further confirms that in AOM coinfection with bacteria and viruses occurs more often than in URI non-AOM. There were no differences in the occurrence of viruses between the groups. However, the AOM patients had more pathogenic bacteria and less non-pathogenic streptococci.

**P1934** Clonality and pilus protein genes of pneumococcal isolates causing acute otitis media in children

A. Vainio, T. Kaijalaainen, R. Siitonen, L. Siira, A. Virolainen (Helsinki, Oulu, FI)

Objectives: Acute otitis media (AOM) is one of the most common infectious diseases among young children in the developed countries and *S. pneumoniae* is still the major bacterial pathogen causing AOM infections. Our aim was to characterise the molecular background of pneumococcal isolates cultured in the middle ear fluid (MEF) and/or nasopharyngeal aspirate (NPA) of the AOM cases.

Methods: The 56 children (age range from 9 months to 6 years and 11 months) with clinically defined AOM were divided into three groups: those with MEF and NPA positive for pneumococci (MEF+/NPA+, N = 19), those with only MEF positive for pneumococci (MEF+/NPA−, N = 34), and those with only NPA positive for pneumococci (MEF−/NPA+, N = 3). All *S. pneumoniae* isolates (N = 75) were studied for antibiotic susceptibility, serotyped by latex agglutination and/or counterimmunoelectrophoresis, and pilus islet 1 (rlrA, rrgC) and 2 (pitaA-sipaA) genes were detected by PCR. In addition, 17 isolates were selected for genotyping by multi locus sequence typing (MLST), based on serotype and pilus islet gene results.

Results: All the 75 pneumococcal isolates were susceptible to penicillin. Among all the isolates, 14 different serotypes were found: 19F (27%), 23F (25%), 6B, 14, 6A, 19A, 11A, 9V, 18C, 24, 33, 3, 15A, and 38, in decreasing order of incidence. The serotype was the same in both MEF and NPA isolates in the same child. Serotypes 9V, 19A, 24, and 33 were found only in the MEF+/NPA+ group and serotypes 3 and 38 only in the MEF−/NPA+ group. Fifteen (20%) of the 75 isolates were detected positive for the pilus islet 1 genes, and they were of serotypes 6B, 6A, 9V, 23F, and 38, in decreasing order of incidence. In MEF+/NPA+ group 8/41 (20%) pneumococcal isolates from four children had pilus islet 1 genes and in MEF−/NPA+ group 7/34 (21%). The pilus islet 2 was not

**P1935** Serotype distribution, antibiotic resistance of strains causing acute otitis media and antibiotic consupption in children in Slovakia

H. Huskova*, J. Trupl, J. Jakubikova, M. Gezo, E. Novakova (Bratislava, Zilina, SK)

Objectives: To assess the distribution of serotype and antibiotics resistance among strains *S. pneumoniae* and *H. influenzae* causing acute otitis media among children <5 year old. To estimate the rates of acute otitis media (AOM)-related ambulatory visits and antibiotic prescriptions patients in Health Insurance.
detected among any of the isolates. The MLST analysis of the selected isolates is currently underway.

**Conclusion:** Pilus 1 gene positivity among pneumococcal MEF and NPA isolates seemed to appear in certain serotypes as reported earlier for invasive isolates. However, the MLST results will reveal the pilus gene association with clonality in more detail.

### P1935 Pneumonia cases in the paediatric intensive care unit of a tertiary-care university hospital

**E. Hajdú**, K. Rácz, G. Tálosi, S. Türi, E. Nagy (Szeged, HU)

**Objectives:** Children patients of pneumonia need ICU admission in cases of respiratory or circulatory failure, pleural effusion, empyema or abscess. Microbiological results can help the effective therapy, especially in cases of nosocomial infections. We evaluate our data of pneumonia patients treated in paediatric ICU from Jan. 01, 2005 to Dec. 31, 2007.

**Methods:** Pneumonia was diagnosed by physical examination, chest X-ray, CT scan, MRI and laboratory examinations. We cultured blood (BACTEC System, Becton Dickinson, Sparks Md), pleural punctions and endotracheal aspirates. Bacteria were identified by conventional methods and VITEK 2 system (bioMérieux, UEtoile, France). The antibiotic susceptibility tests of isolates were determined by using disk diffusion method, E-test and the breakpoints recommended in the NCCLS/CLSI guidelines. RSV antigen was detected by immunochromatography.

**Results:** Our results are summarised in the table.

<table>
<thead>
<tr>
<th>Patients’ data</th>
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</thead>
<tbody>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>31</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>23</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>1 month to 20 years (mean 7.4 months)</td>
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<tr>
<td>Diagnosis</td>
</tr>
<tr>
<td>Pneumonia</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>Pneumococci</td>
</tr>
<tr>
<td>31</td>
</tr>
<tr>
<td>Other chronic disease</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>Severe maternal retardation</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>Congenital ventricular cardiomyopathy</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Gastro-esophageal reflux</td>
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<tr>
<td>4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>Status post renal transplantation</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>Asthma bronchial</td>
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<tr>
<td>2</td>
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<tr>
<td>Morbus Down</td>
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<tr>
<td>1</td>
</tr>
<tr>
<td>Admission</td>
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<tr>
<td>From home</td>
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<tr>
<td>19</td>
</tr>
<tr>
<td>From other hospital</td>
</tr>
<tr>
<td>70</td>
</tr>
<tr>
<td>From other ward of Department of Paediatrics</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Antibiotic use before admission</td>
</tr>
<tr>
<td>46(55%)</td>
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<tr>
<td>Length of stay in ICU</td>
</tr>
<tr>
<td>2–48 days (mean 7.4 days)</td>
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<tr>
<td>Antibiotic therapy</td>
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<tr>
<td>Ceftazidim</td>
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<tr>
<td>23</td>
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<tr>
<td>Pencillin</td>
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<tr>
<td>11</td>
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<tr>
<td>Macrolides</td>
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<tr>
<td>10</td>
</tr>
<tr>
<td>Aminoglycosides</td>
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<tr>
<td>5</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole (suspected Pneumocystis carini infection)</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Glycopeptide</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>Carbapenems</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>Lofutidine eye</td>
</tr>
<tr>
<td>5.5%</td>
</tr>
<tr>
<td>Aortitis</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>Haemophilus influenza</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Pseudomonas aeruginigena</td>
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<tr>
<td>4</td>
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<tr>
<td>Staphylococcus aureus</td>
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<tr>
<td>Klebsiella pneumonia ESBL</td>
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<td>Chlamydia pneumonia</td>
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<td>Candida lusitana</td>
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<td>Angiobes</td>
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<td>RSV</td>
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<td>3</td>
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<td>Pathogen not identified</td>
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<td>28</td>
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**Conclusions:** Despite the high rate of pre-treated patients, in 48% of the cases the pathogens could be identified. All three children lost at the ICU had other severe chronic diseases. Our study indicates the importance of microbiological diagnosis in finding efficient treatments.

### P1936 Clinical evaluation of a new commercial PCR-DNA microarray system for simultaneous detection of 17 respiratory viruses in French infants hospitalised for acute bronchiolitis

**L. Andreoletti**, A. Huguenin, L. Moutte, M. Abely, K. Bessaci, F. Renois, T. Nenninger, F. Currat, N. Leveque (Reims, FR)

**Background:** Rapid testing for viral infection is recommended in infants who require admission to hospital with acute bronchiolitis in order to guide cohort arrangements or to improve therapeutic management in case of respiratory distress. Clinical virology laboratories used traditional methods as direct fluorescent-antibody assay (DFA) and culture for the detection of six or seven conventional respiratory viruses. In the present study, a new commercially available assay using RT-PCR followed by microarray detection assay designed for detection of 17 pathogenic respiratory RNA viruses was evaluated by testing clinical samples of infants hospitalised for bronchiolitis.

**Methods:** From October 2007 to December 2008 we retrospectively selected 65 infants (mean age: 3.5 months, SD: 3.2 months) admitted to the paediatric department (University Hospital Center of Reims, France) for acute bronchiolitis. Infants with congenital heart disease or with a chronic genetic or acquired immunodepression were excluded. Nasopharyngeal aspirate samples of the selected patients were tested by DFA and cell-culture detection assays and by microarray detection assay (Clart Pneumovir Version 3.0, Genomica, Madrid, Spain) for the presence of respiratory viruses.

**Results:** One or more potential causative viral agents were detected in 47, 51, 63 of 65 samples by viral culture, DFA and the microarray detection assay, respectively (P<0.01). The frequency of detection of conventional respiratory viruses appeared to significantly higher using the microarray assay than using the classical techniques (P=0.02). The Pneumovirus assay detected 43 mixed infections that the most common associations were: adenovirus and RSV (26%), bocavirus and RSV (23%) and metapneumovirus and RSV (23%). Mixed infections appeared not to be statistically associated with the severity of the disease or secondary hospitalisation events for acute bronchiolitis or asthma within 6 months after the time of inclusion.

**Conclusion:** The use of this PCR-DNA microarray system in clinical virology practice allows a rapid and accurate detection of conventional and newly discovered viral respiratory pathogens in infants hospitalised for acute bronchiolitis. Moreover this new assay would be of major interest for the development of future therapeutic and preventive strategies to fight against the viral causes of bronchiolitis.

### P1937 Detection of atypical bacterial and viral antibodies in lower respiratory tract infections in children by use of an immunofluorescence method

**K. Papavasileiou**, H. Papacastanis, I. Varzakakos, S. Chatzianagiou, A. Voyiatzi (Athens, GR)

Lower respiratory tract infections (LRTIs) are the leading cause of morbidity and mortality among children. Most commonly leads to hospitalisation due to the frequent relapses of the disease.

**Objective:** The aim of the study was the detection of antibodies (IgM, IgG) of viruses and atypical bacteria in children with lower respiratory tract infections during the period October 2007–April 2008.

**Methods:** Sera were collected from a total of 100 hospitalised children, aged 2 months to 14 years, with LRTIs in acute and convalescent-phase. We used the indirect immunofluorescence method (pneumoslide immunofluorescence method (pneumoslide IgG, IgM – VIRCELL)) in order to detect simultaneously specific (IgM, IgG) antibodies against 4 atypical bacteria (L. pneumophila, M. pneumoniae, C. burnetii, C. pneumoniae) and 4 viruses (Adenovirus, RSV, Influenza A, Influenza B).

**Results:** Antibodies against viral or atypical bacteria were detected in 88 out of 100 children (88%) with LRTIs. A single pathogen was identified in 38 children (43%). Mixed infections were found in 50 children (57%): RSV + Adenovirus (20), RSV + Adenovirus + M. pneumoniae (15), M. pneumoniae + Adenovirus + Influenza B (5), RSV + Influenza A

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*S565* Paediatric infections
Colonisation of intravascular catheters in the intensive care unit of a paediatric hospital during a ten-year period (1999–2008)

K. Papavasileiou*, H. Papavasileiou, G. Oikonomopoulos, C. Ilidou, A. Voyatzis (Athens, GR)

Intravascular catheters (IVC) are indispensable in modern-day medical practice, particularly in intensive care units (ICUs). Although such catheters provide necessary vascular access, their use puts patients at risk for local and systemic infectious complications.

Objective: To study the rate of colonisation of intravascular catheters, the frequency of appearance of the pathogens, as well as their susceptibility pattern to various antimicrobial agents.

Methods: During a ten year period (1999–2008) we performed routine examination of the tips of 413 IVCs. The catheters were cultured by the Maki semi quantitative method and all samples, after enrichment in cooked meat broth, were subculture on selective media. All pathogens were identified by classical microbiological methods and susceptibility to antibiotics (MIC) was determined with the automated system VITEK 2 (BioMérieux, France), according to CLSI recommendations.

Results: Out of the 413 catheters examined, 247 (60%) were sterile, while 166 (40%) were colonised. We found 198 pathogens. In 80.7% of catheters one pathogen was isolated, in 14.5% two and in 4.8% three or more. Gram+ cocci (126, 63.6%) were most frequently isolated, followed by Gram− bacteria (34, 17.2%), fungi (25, 12.6%) and finally Gram+ bacteria (13, 6.6%). Coagulase negative Staphylococci (CNS) (105, 53%) were the dominant isolates, followed by fungi (25, 12.6%), Enterococcus spp (12, 6%), Acinetobacter spp (11, 5.5%) and S. aureus (7, 3.5%). From the 105 CNS, 54.2% were resistant to methicillin, 60% to erythromycin, 45.7% to clindamycin, 38% to gentamicin, 25.7% to ciprofloxacin, 10.5% to tetracycline and 35% to netilmicin. There was no resistance to levofloxacin, linezolid and vancomycin, whereas one strain showed intermediate susceptibility to teicoplanin. Multiresistance was detected in Acinetobacter baumanii strains. 52% of patients with positive blood culture had catheter-associated bacteraemia.

Conclusions: The methicillin resistant CNS is the most frequent cause of colonisation of intravascular catheters in ICU. Increased resistance to antibiotics is observed. Physicians should observe the international recommendations that are designed to reduce the infectious complications associated with intravascular catheter use.

Prevalence and antimicrobial susceptibility among Pseudomonas aeruginosa strains isolated in a paediatric hospital

A. Makri*, K. Kalimeratzi, G. Oikonomopoulos, E. Nika, C. Ilidou, A. Voyatzis (Penteli, GR)

Objectives: to evaluate the prevalence and to investigate the antimicrobial resistance patterns of Pseudomonas aeruginosa (Pa.) strains isolated from hospitalised children with severe infection during a four year period (2005–2008).

Material and Methods: Totally, 472 Pa. strains were isolated from hospitalised children in Surgical clinics (60.8%), Paediatric clinics (24.2%), Intensive Care Unit (ICU) (10.8%) and from outpatients (4.2%). Identification and susceptibility patterns were performed by the automated system Vitek 2 (bio-Mérieux). Metallo-β-lactamases (MBL) production was screened by E-test MBL-stripe (AB-Biodisk) and by double disk synergy test IMP/EDTA.

Results: The origin of the 472 studied isolates was: ear smears 39.8%, low respiratory tract secretions 25%, wound specimen 21.8%, urine 12.2% and blood specimen 2.1%. Resistance rates of Pa. strains to 12 antibiotics ranged: ticarcillin (TIC) 33%, netilmicin (NET)18.9%, aztreonam(ATE) 14.1%, piperacillin (PIP) 14%, cefazidime (CAZ) 13.5%, gentamicin (GM) 10.3%, ciprofloxacin (CIP) 10.3%, ticarcillin/CA (TIM) 10%, tobramycin (TOB) 9.9%, amikacin (AN) 8.5%, imipenem (IMP) 6% and piperacillin-tazobactam (TZP) 4.7%. Most of Pa. isolates from urine was susceptible to the majority of the antibiotics, expressing a low rate resistance to AZT (4.7%) and TIM (4.7%). P aeruginosa isolates from bronchopulmonary infections showed a significant resistance to TIC (33%), PIP (10%), CAZ (10%) and AZT (9%) due to chromosomal resistance and the production of extended spectrum β-lactamases (ESBL) plasmid. Multiple antibiotics resistance (>5 antibiotics) was observed to the majority of isolated Pa. strains from ICU samples while resistance rate to carbapenems was 11.1% (MIC50 >5mg/l).
Anaerobic bacteriology and antibiotic susceptibility of intra-abdominal community-acquired infections in children

A. Makri*, C. Iliadou, K. Kalimeratzi, G. Oikonomopoulos, A. Pantazatou, A. Aelami, A. Voyatzis (Pentieli, Athens, GR)

Objectives: To investigate the incidence and the antibiotic susceptibility of anaerobes isolated in complicated intra-abdominal (cIAIs) and postoperative wound infections (PSWIs) in paediatric patients.

Material-Methods: Retrospective data for bacterial clinical specimen from 432 hospitalised children with intraabdominal and surgical wound infections over a two year period were analysed. Cultures were performed and confirmed by the Greek National Centre of Gram negative anaerobe bacteria.

Results: From 303 examined positives cultures (70%) were recovered aerobes 64.5%, anaerobes 5.3% and mixed aerobes and anaerobes 30%. The anaerobes bacteria isolated were: Bacteroides fragilis group (Bacteroidales-32%), Peptostreplococci (28%), Clostridium spp (14%), Prevotella spp. (8%), Bacteroides capillosus (6%), Fusobacterium spp (5%), Veillonella spp (4%) and Porphyromonas spp (3%). Bacterodes fragilis group and Peptostreptococci predominated more often in abdominal pus samples, while Clostridium spp. and Prevotella spp. were associated with PSWIs. Out of Bacteroides fragilis group, Bacterodes fragilis was the most recognised anaerobic pathogen in appendicitis-related infections accounted for 15%. Clostridium perfringens (10%) was specially found in cases with gangrenous appendicitis. All strains of Clostridium spp were susceptible to the antibiotics tested while Porphyromonas spp and Prevotella spp showed a resistance rate 33% and 25% to clindamycin respectively (high MIC >256 mg/l). All Bacteroides fragilis group isolates were susceptible to imipenem but a significant increase in resistance was observed to clindamycin (28.5%, high MIC >256 mg/l). All Bacterodes fragilis group isolates were susceptible to metronidazole (70 monoinfections, 26 virus-virus infections and 10 virus-bacteria coinfections), adenoviruses in 162 (3.5%) cases (142 monoinfections, 16 virus-virus confections and 4 virus-bacteria coinfections), astroviruses in 108 cases (2.3%) (70 monoinfections, 26 virus-virus infections and 12 virus-bacteria) and noroviruses in 185 cases (4.02%) (all monoinfections). Although intensive efforts at laboratory confirmation were attempted in our study, an enteropathogen was found in approximately half of the cases. In conclusion, even though Rotavirus group A was the leading cause of acute gastroenteritis with the most significant role in hospitalised children with severe diarrhoea in Greece, further studies will be necessary to augment our knowledge in the aetiology of enteric infections, which will be helpful in the rational application of effective vaccines.

Infectious complications in paediatric acute liver failure, a single-centre experience

G. Godbole*, A. Dhawan, N. Shanmugam, A. Verma (London, UK)

Objective: Acute liver failure (ALF) is rare in children but carries high mortality. Infectious complications in adults are considered to be an important cause of mortality, however there is lack of data in paediatric population. The aim was to determine the incidence of infectious complications (IC) and their effect on outcome in children with ALF.

Methods: Retrospective review of the case records of children presenting with ALF. All patients had surveillance of cultures from all sterile body fluids every week or more often if clinically indicated. All received antimicrobial prophylaxis with amoxicillin, a cephalosporin, an antifungal, fluconazole or amphotericin B (if on assisted ventilation) and high dose acyclovir in neonates. Biochemical parameters of liver dysfunction, renal dysfunction, duration of hospital stay and patient outcome were compared between patients with IC and non-infectious groups.

Results: 145 children (69 male), median (range) age of 4.22 (1 day-16 yrs) years were studied. The aetiology of ALF included paracetamol overdose, viral infections, metabolic, indeterminate, autoimmune, neonatal hemochromatosis and others. 47/145 patients had proven IC (32%). 18 episodes of bacteraemia were observed in 13 patients, the most common organism was Enterococci spp. Lower respiratory tract infection was seen in 12 patients and most common organism was Pseudomonas spp. Urinary tract infection (UTI) was also seen in 12, Candida albicans contributed to more than half of the UTI cases. IC occurred in patients after a median (range) duration of 14 days (0–54 days) of admission. 8 (5%) had IC at admission. Median (range) duration of hospital stay in patients with IC 35(4-201) days was significantly higher than those without IC 11 (1–14) day, p<0.0001. The duration of ventilation was also significantly higher in the group with IC (10 days) as compared to non-infectious group (5 days); p<0.01. 45% (21) in IC group had liver transplantation while as compared to only 26% (26) in non-infectious group (P<0.03). Overall mortality was 16% (23) of which 5% (8) were from IC group, 10% (15) from non infectious group, the difference was not statistically significant.

Viral, bacterial and parasitic aetiology of acute gastroenteritis in hospitalised children in north-western Greece


The aim of this study was to determine the aetiology of acute diarrhoea in hospitalised children under 5 years of age in Northwest Greece and to improve knowledge of the aetiology of gastrointestinal pathogen using traditional and molecular diagnostic techniques. From 2000 to 2006, faecal samples from 4604 children under 5 years old (median age 14 months) were collected from five hospitals in NW Greece. Various common bacteria and parasites associated with diarrhoea were investigated by conventional techniques. Viral agents were investigated by antigen detection [e.g. rotavirus (Group A), adenovirus, astrovirus and norovirus] using an enzyme immunoassay (IDEIA; DAKO Cytomation, Angel Grove, UK) and molecular methods [e.g Rotavirus using an “in house” RT-PCR and Adenovirus using a PCR-microplate hybridisation assay (PCR Adenovirus Consensus, Argene, Biosoft, France)]. From 4604 stool samples examined, aetiologic agents were detected in 1789 cases (38.9%). Monobacterial infections were detected in 389 (21.8%) cases (Salmonella spp. in 258, Shigella spp. in 5, Campylobacter jejuni in 118, Escherichia coli in 2, Aeromonas hydrophila in 2), while single viral infections were identified in 1338 children (74.8%). Viral-bacterial confection was found in 26 (1.4%) cases and viral-viral confection in 36 (2%) cases. No sample was positive for parasites. Viral pathogenes were identified in 1400 children (30.4%): Group A rotavirus was detected in 983 (21.35%) cases (941 monoinfections, 32 virus-virus confections and 10 virus-bacteria confections), adenoviruses in 162 (3.5%) cases (142 monoinfections, 16 virus-virus confections and 4 virus-bacteria confections), astroviruses in 108 cases (2.35%) (70 monoinfections, 26 virus-virus infections and 12 virus-bacteria) and noroviruses in 185 cases (4.02%) (all monoinfections). Although intensive efforts at laboratory confirmation were attempted in our study, an enteropathogen was found in approximately half of the cases. In conclusion, eventhough Rotavirus group A was the leading cause of acute gastroenteritis with the most significant role in hospitalised children with severe diarrhoea in Greece, further studies will be necessary to augment our knowledge in the aetiology of enteric infections, which will be helpful in the rational application of effective vaccines.
**Conclusions:** Incidence of culture proven sepsis and mortality associated with it is low in our experience. Breakthrough sepsis is more common hence a more stringent surveillance should be employed in whom the duration of hospital stay is more than two weeks.

**P1944** Low-dose erythromycin in the treatment of gastro-oesophageal reflux disease in infants, A. pilot, randomised, double-blind, placebo-controlled trial

A. Banackiewicz, A. Gawrońska, P. Albrecht, A. Radzikowski (Warsaw, PL)

**Objectives:** Gastro-oesophageal reflux disease (GERD) is defined as symptoms or complications of reflux of gastric content into the oesophagus. Commonly used medications for the treatment of GERD include acid-suppressant and prokinetic drugs. Based on systematic review, the literature supports the use of erythromycin as a prokinetic agent, however most reviewed trials were conducted in preterm infants. The aim of this study was to assess the effectiveness of low-dose erythromycin in the treatment of gastro-oesophageal reflux disease (GERD) in full-term infants.

**Methods:** This was a double-blind, randomised, placebo-controlled clinical trial. Subjects aged 1–11 months with symptoms of GERD confirmed both by 24-hour pH-monitoring and the Infant Gastrooesophageal Reflux Questionnaire Revised (I-GERQ-R) were randomly assigned to receive either erythromycin at dose 1–3 mg/kg/dose or a comparable placebo. Both the active treatment and placebo were taken orally three times daily (20 minutes before meals) for 2 weeks. Parents were asked to fill out the I-GERQ-R at the study entry and at 2 and 4 weeks after enrollment.

**Results:** 24 infants were enrolled in the study, 12 in erythromycin group and 12 in placebo group; no significant difference was found between two groups. Difference in the I-GERQ-R score in erythromycin group compared with placebo group was statistically significant reduced (p = 0.000003) between beginning therapy and 4 weeks but was not between beginning therapy and 2 weeks (p = 0.07).

**Conclusions:** Erythromycin at dose 1–3 mg/kg/dose given three times daily was effective in treating GERD in full-term infants. Its effect seemed to be time-dependent.

**P1945** Vesicoureteral reflux in children with suspected and proven urinary tract infection

A. Hannula*, M. Venhola, M. Renko, T. Pokka, N.P. Huttunen, M. Uhari (Oulu, FI)

**Objectives:** The clinical significance of vesicoureteral reflux (VUR) has been challenged. We wanted to estimate the prevalence of VUR and clinically significant ultrasonography (US) abnormalities in a large unselected group of children with proven and suspected UTI and even without UTI.

**Methods:** We reviewed the reports of renal or abdominal US and voiding cysotrography (VCUG) during an 11-year period. Altogether 8567 patients; 2145 patients had imaging studies performed because of UTI and 2036 patients (1481 girls, 555 boys) under the age of 15 years fulfilled the inclusion criteria. Based on the age of the urine cultures we classified the reliability of UTI diagnoses into 5 classes (proven, likely, unlikely, false and no data). The mean age of the children was 3.2 years (SD 2.9). Renal US was performed on all children and VCUG was performed on 1185 (58%) children.

**Results:** Vesicoureteral reflux was found in 405/1185 (34.2%) children and in 181 (15.0%) VUR was grade III to V. The prevalence of VUR was similar among those with proven and false UTI (37.4% vs. 34.8%), relative risk (RR) 1.08, 95% CI 0.7 to 1.7, p = 0.75. There was a significant negative trend in occurrence of VUR with increasing age (p = 0.001). Ultrasonography was abnormal in 422/2036 (20.8%) cases.

Clinically significant US abnormalities occurred in 87/583 (14.9%) of patients with proven UTI and significantly more seldom in 11/145 (7.6%) of patients with false UTI diagnosis (RR 1.96, 95% CI 1.1 to 3.5, p = 0.02).

**Conclusion:** The prevalence of VUR was similar in all patient groups irrespective of the diagnostic reliability of UTI and decreased with increasing age. On the contrary, the frequency of significant US abnormalities increased as the reliability of UTI diagnosis improved. This supports the claim that VUR is not significantly related to UTI and its occurrence even among healthy children is significantly higher than the traditional estimates. We suggest that routine search for VUR with VCUG after UTI cannot be recommended.

**P1946** Risk factors predicting recurrent urinary tract infection in childhood

M. Mikelsaar*, I. Vainumäe, K. Trusula, E. Sepp, S. Kõljalg (Tartu, EE)

The urinary tract infection (UTI) is one of the most common recurrent bacterial diseases in childhood. However, the role of clinical and microbiological risk factors in the development of recurrent UTI has not been evaluated applying novel molecular methods.

**Aim:** to assess if patient age, recurrent UTI causing microbiological agent, clinical presentations and antimicrobials administered in the treatment predict the recurrences of UTI in children.

**Methods:** Prospectively, 64 patients (1 month–15 years) with first acute pyelonephritis were conducted for evaluation of clinical and laboratory data during a year. Urine cultures, antibiotic susceptibility of the agent, genotyping by randomly amplified PCR (RAPD-PCR) and pulsed field gel electrophoresis (PFGE) of 78 consecutive Escherichia coli isolates from 27 patients with recurrent UTI were performed.

**Results:** Half (32/64) of the patients experienced recurrences (79 episodes, 2.5 ± 1.5 per patient) and the majority (62%) of recurrent episodes were asymptomatic. Vescoureteral reflux was found in 22% (14/64) of patients. The majority (55/64, 86%) of first acute pyelonephritis and recurrent episodes (73/79, 92%) were caused by Escherichia coli.

E. coli was initially resistant to ampicillin (33%), trimethoprim-sulfamethoxazole (41%) and both (23%). There were no differences in resistance to β-lactams between initial and consecutive E. coli isolates. In more than half of the 21/27 patients (78%) the unique genotypically identical E. coli strains caused the UTI relapses indicating the persistence of the particular strain. Individual risk factors predicting a complicated course of UTI were age over 2 years (OR 4.19, CI 4.12–12.37, p = 0.01) and initial β-lactam treatment (OR 5.9, CI 1.5–20, p = 0.01, adjusted for gender and age).

**Conclusion:** Genotypically identical E. coli strains with stable β-lactam susceptibility in patients at the age over 2 years treated initially with β-lactams predict the recurrent UTI in childhood as these antimicrobials may not reach the intracellular clones of persisting E. coli.

**P1947** Children's urinary pathogens in a Greek hospital, 2007–2008

L. Zachariadou*, M. Kyprais, M. Ziga, M. Chondrogianni, A. Pangalis (Athens, GR)

**Objectives:** To present the frequency of urinary tract infection pathogens in children, as well as the differentiation in isolation rates of species, among in and out patients.

**Methods:** 28,313 urine specimens were examined from January 2007 to December 2008. 20,814 samples derived from in and 7,499 from out patients, either visiting the emergency unit or the special nephrology department, on a monthly follow up program, due to urinary tract congenital anatomic or functional abnormalities. Samples were mainly collected by suprapubic aspiration, in and out catheterisation, or clean-catch midstream or “bagged” specimen, depending on the age of the little patients. The diagnosis of urinary tract infection was based on precise clinical and laboratory criteria according to the European guidelines and in case of doubt the culture was repeated.

**Results:** 2,476 (8.7%) cultures were positive with one causative pathogen and only 45 (0.16%) with two and 2 with three pathogens. As expected, the dominant pathogen in all isolates was E. coli (70.7%), followed by P. mirabilis (8.4%), K. pneumoniae (6.6%), Entercococcus...
Outcome of acute childhood bacterial meningitis in Luanda, Angola

T.I. Pelkonen*, M. Leite Cruziero, L. Monteiro, A. Pitukántana, I. Roine, H. Peltola (Luanda, AO; Lisbon, PT; Helsinki, FI; Santiago, CL)

Objectives: Little reliable information on the outcomes of bacterial meningitis (BM) of childhood is available from sub-Saharan Africa, although the incidence is highest there. We report data from a large prospective study in Luanda, Angola.

Methods: The series comprised all children who presented at the Luanda Children's Hospital with suspected BM in the 36-month period of 2005–08. Our three outcome measures were death, severe neurological sequelae (SeNeSe) comprising blindness, quadriplegia, hydrocephalus, or severe psychomotor retardation, and deafness (better ear's threshold >80 dB).

Results: Hearing was measured using brain stem auditory evoked response.

Conclusions: The series comprised all children who presented at the Luanda Children's Hospital with suspected BM in the 36-month period of 2005–08. Our three outcome measures were death, severe neurological sequelae (SeNeSe) comprising blindness, quadriplegia, hydrocephalus, or severe psychomotor retardation, and deafness (better ear's threshold >80 dB).

Objective: To describe the epidemiologic features, clinical presentation, bacteriological and therapeutic findings, spectrum of complications and outcome in children with invasive meningococcal disease (IMD) admitted to the Hospital of Infectious Diseases of Iasi during ten years.


Results: From 1998 through 2007, 225 cases of IMD were admitted to the Hospital of Infectious Diseases of Iasi; most of them, 191 (85%), occurred in children. Clinically, 79% of the cases presented with meningitis predominantly and 21% had sepsis (+/-) meningitis. We had an average of 19 patients for each year (range, 10–26 patients). Most of the patients (70%) were from rural communities. The highest incidence (60%) of cases was recorded in the late winter and early spring months. The male to female ratio was 1:1. Half of patients had less than 2 years old. The median age of patients was 24 months (range, 3 weeks to 18 years). Clinical manifestations included meningitis and 60 patients (79%) as sepsis, 36 (19%), seizures in 28 (15%), respiratory failure in 20 (10%), septic arthritis in 3 (1.6%). Microbiological confirmation was based on direct microscopic examination after Gram staining in 51.5% of the cases, culture in 36%, and detection of soluble antigens in cerebrospinal fluid in 34.5%. Serogrouping was available on 95 (50%) of the patients. Serogroup B was isolated in 46 (48%) of 95 patients. The clinical form was severe in 40 cases (21%). Unfavourable outcomes occurred in 24 of 191 patients, including a mortality rate of 11%. Among the survivors, three patients had hearing loss. Of the 77 (40.3%) of all cases was treated before admission, 60 (77.9%) of them receiving preadmission antibiotic therapy, early diagnosis is sometimes difficult; that's why laboratory confirmation should be improved by the introduction of PCR-based techniques.

Objective: Narrow spectrum penicillins have been shown to have least impact on bowel colonisation in neonates. The most widely recommended empiric regimens of penicillin or ampicillin combined with gentamicin have never been compared in these terms.

We aimed to compare the ampicillin with penicillin in term of influence on bowel colonisation by opportunistic microorganisms including ampicillin resistant Enterobacteriaceae in neonates with suspected early onset neonatal sepsis and to identify risk factors interfering with colonisation process.

Methods: A cluster-randomised two-centre switch-over study included all neonates hospitalised from August 2, 2006 until November 30, 2007.

Results: Each treatment group included 139 neonates. Compared to penicillin the mean TCD of Klebsiella pneumoniae and Candida spp. was higher than that of K. oxytoca, Acinetobacter baumannii, Enterococcus spp, Streptococcus spp and S. aureus was lower in the ampicillin group. The ratio of patient colonised by ampicillin resistant Enterobacteriaceae in both treatment groups was similar. Multivariate logistic regression analysis at base identified ampicillin as an independent factor favouring colonisation with K. pneumoniae (OR 2.41; 95% CI 1.1−5.27) and preventing that with S. aureus, enterococci and ampicillin resistant A. baumannii (OR 0.24; 95% CI 0.09−0.68; OR 0.48; CI 95% 0.27−0.87 and OR = 0.11; 95% CI 0.002−0.78, respectively).

Objective: Ampicillin or penicillin for empiric treatment of early-onset neonatal sepsis: influence on bowel colonisation by aerobic and facultative anarobic bacteria

Y. Purn*, E. Sepp, T. Metsvaht, M.L. Ilmoja, H. Pisarev, M. Pauskar, I. Lutsar (Tartu, EE)

Methods: The present criteria of acute BM were met by 522/680 (77%) patients with the median age of 11 months. The median length of illness before admission was 4 days, and 68% had had convulsions. No less than 88% had received some medication, 41% antimicrobials. On admission, consciousness was impaired in 71%, focal neurological signs were present in 88% had received some medication, 41% antimicrobials. On admission, 60(77.9%) of them receiving preadmission antibiotic therapy, early diagnosis is sometimes difficult; that's why laboratory confirmation should be improved by the introduction of PCR-based techniques.

Objective: Ampicillin or penicillin for empiric treatment of early-onset neonatal sepsis: influence on bowel colonisation by aerobic and facultative anarobic bacteria

Y. Purn*, E. Sepp, T. Metsvaht, M.L. Ilmoja, H. Pisarev, M. Pauskar, I. Lutsar (Tartu, EE)
**Conclusion:** Ampicillin and penicillin when combined with gentamicin are associated with different bowel colonisation pattern by individual microorganisms but neither regimen appears to have a clear disadvantage of out-selecting ampicillin resistant Enterobacteriaceae.

**P1951 Strategies for the sequence study of the exposure to pulmonary tuberculosis in a neonatal unit**

*C. Moler*, A. *Mirada, R. Font, M. Olmo, L. Canales, J.L. de Marco* (Terrassa, ES)

**Objectives:** Almost the 50% of newborns exposed to tuberculosis disease develop lung disease and 20% can develop disseminated disease/meningitis. On the other hand, tuberculin skin test (TST) has very low sensitivity before 6 month age. The recommended strategy in this population is to start an empirical treatment early. The aim of our study is to assess the incidence of infection/latent disease in a cohort of newborns exposed to a smear-negative nurse with tuberculosis lung disease (culture positive in samples obtained by bronchoscopy) in a neonatal unit, as well as to describe the diagnostic and therapeutic management.

**Methods:** All the newborns exposed at least 1 day to the index case in the 3 previous months to her diagnosis (January–April 2008) were investigated. The study strategy consisted in a TST, blood analysis at inclusion and 1 month after to exclude adverse effects and chest radiograph (Rx). Chest Tomography (CT) and Quantiferon were performed in case of misdiagnosis. Once tuberculosis disease was ruled out, cases initiated prophylaxis with isoniazid orally (10 mg/kg/d) up to 6 months of age, then a second TST was performed. TST was assessed on all healthcare workers.

**Results:** In the study period, 60 newborns were exposed to the index case. The patients were included and studied in a period of 3 weeks. The TST was negative at the time of inclusion and at 6 months in all cases (1 death for underlying pathology before the second TST). One case had abnormal Rx, with subsequent CT and Quantiferon normal tests. Of 114 neonates with positive eyes, 53 (88.6%) patients accepted to start prophylaxis; in 2 (3%) prophylaxis was contraindicated for underlying pathology and in 5 (8%) there was negative by parents. Blood analysis one month after inclusion was performed in 47 out of 53 (88.6%) newborns in isoniazid treatment, and in only one case (2%) prophylaxis was stopped for hypertransaminemia. 78% of the contacts finished prophylaxis. 5 patients (9%) discontinued prophylaxis by decision of the parents. After 9 months of follow up, no cases of tuberculosis are reported regardless of having done prophylaxis.

**Conclusions:** Given the limited bibliography, the small size of our sample and the potential severity of the disease we feel justified the prophylaxis until 6 months age and close follow up of the exposed cases. The realisation of TST at the time of inclusion and at 6 months age, as well as chest radiograph, seems to be a valid strategy to exclude the disease in this population.

**P1952 Childhood brucellosis in northern Greece**

*D. Raillas*, O. *Tsatsiou, O. Karamitou, M. Spanidou, A. Kanszczidou, J. Kacalistas* (Thessaloniki, GR)

**Objectives:** Human brucellosis is a common zoonotic infection worldwide. Greece is one of the countries of the European Union with high incidence of the disease. The aim of this study was to evaluate the epidemiological, clinical and laboratory characteristics of brucellosis in children hospitalised in our Department.

**Methods:** We evaluated the records of 164 children, up to 14 years old, with brucellosis, who were hospitalised in our Department from 1980 to 2008. Inclusion criteria were: 1) clinical picture compatible with brucellosis, 2) Wright seroagglutination test positive with titers 1:160 or above 3) blood culture positive for brucella, 4) PCR positive for brucella. Patients who fulfilled two or more of the above criteria were included in the research.

**Results:** The 68% of the patients were males. The median age of children was 8.5 years (2 months – 14 years). Consumption of unpasteurised milk or dairy products was reported to 47% of patients, whereas 44% of the cases were related to other intrafamiliar instances. Fever was the most common symptom, (81%), with mean duration of 16 days. Other manifestations included arthralgia (59%), night sweets (43%), hepatomegaly (57%), splenomegaly (35%), lymphadenopathy (11%) and limp (12%). Brucella spp. was isolated in 30% of the patients. The course of the disease was good in the majority of patients. Complications were observed only in 3%: meningitis (2), pneumonia (1) and osteochondritis (2). The treatment schedule we used was doxycycline p.o. for 21 days plus streptomycin IM for 14 days. Recurrences were noted in 7.5% of the patients. In most cases the main symptom was fever of short duration.

**Conclusion:** Childhood brucellosis remains a common health problem in Greece. The disease manifests itself mostly mildly in children, with a low rate of complications. The treatment with doxycycline plus streptomycin is very effective with a recurrence rate that is lower than that of other therapeutic schedules, despite the shortness of the course. However, because this duration is shorter than that of official guidelines, perhaps it needs a more extended study for a full evaluation.

**P1953 Study of bacterial conjunctivitis in neonates with special reference to Chlamydia trachomatis**

*S. Tabatabaei*, A. *Afjee, A. Karimi, F. Fallah, N. Tahami Zanjani* (Tehran, IR)

**Background:** Conjunctivitis is the most common eye disease of newborns. Conjunctivitis during the neonatal period is usually acquired. The most common bacterial infections can cause serious eye damage are gonorrhea (Neisseria gonorrhoeae) and Chlamydia (Chlamydia trachomatis), which can be passed from mother to child during birth. The incidence of gonococcal ophthalmia neonatorum decreased in industrialised countries secondary to widespread use of silver nitrate prophylaxis and prenatal screening and treatment of maternal gonorrhoea. In comparison, chlamydial trachomatis is the most common organism causing ophthalmia neonatorum in the USA. This study is to identify the prevalence of the causative agents of ophthalmia neonatorum, chiefly *Chlamydia trachomatis* in two hospitals (mofid & mahdieh) on 2007–2008.

**Methods and Materials:** We will study 114 neonates with conjunctivitis in first 4 weeks of life. We obtain two swab specimens containing epithelial cells of conjunctiva. Laboratory diagnosis was based on bacterial culture and Gram staining. The isolated bacteria were identified using standard procedures. For identifying *Chlamydia trachomatis* we will use cell culture (gold standard) and Giema staining.

**Results:** Of 114 neonates with positive eye swab or conjunctival scraping cultures, *Chlamydia trachomatis* was the second most common (n = 17, 14.9%) cause of acute neonatal conjunctivitis after coagulase-negative staphylococci (n = 86, 51%). Bacterial cultures were negative in 23.1% of neonates despite clinical signs of conjunctivitis. The median age of positive *Chlamydia trachomatis* neonates was day 9 of life (range, day 1–30).

**Conclusion:** Based on previous studies, prevalence of *Chlamydia trachomatis* in newborns was 6–21%. This prevalence is 14.9% in our study. *Chlamydia trachomatis* was the second most common causative organism in acute neonatal conjunctivitis. Gram Positive Cocci were the most common cause of bacterial infections. Therefore we recommend doing giemsa staining for the characteristic intracellular plasmic inclusions and tissue culture techniques for the organism from a conjunctival swab.

**P1954 Epidemiology of bacterial hand infections in a paediatric population**

*M. Matsas, N. Paleologou, M. Papadimitriou, A. Kallimani, E. Lebessi* (Athens, GR)

**Objectives:** Hand infections pose difficult diagnostic problems because of the wide microbiology and anatomy involved. The aim of this study was to evaluate the bacteriological spectrum and the antimicrobial susceptibility patterns in infants and children with bacterial hand infections, referred for care at “P & A. Kyriakou” Children’s Hospital.
**Methods:** All specimens from hand infections operated on in the outpatient clinic and the department of orthopedics from January 1, 2007 to October 31, 2008, were reviewed retrospectively, using the laboratory archives and the patient charts. Culture of specimens and identification of bacteria were performed by conventional methods. Antimicrobial susceptibilities of isolates were determined by disk diffusion method according to the CLSI guidelines.

**Results:** Totally, 142 specimens from an equal number of children [82 boys (56%) and 60 girls (44%)] were recorded. The median age of the patients was 5.5 years (range 9 months to 14 years). Most of them were presented with paronychia. One to five bacteria were isolated from 113 specimens (79.6%), while in 29 (20.4%) there was no isolation of any bacterial pathogen. The majority of infections were monomicrobial (69/113, 61%), with most frequently isolated bacteria Staphylococcus aureus (49/69, 71%) and Streptococcus pyogenes (18/69, 26%). In mixed infections (44/113, 39%) the most frequently isolated bacteria were also S. aureus (31/44, 70%) and S. pyogenes (21/44, 48%), followed by anaerobes (15/44, 34%) and Eikenella corrodens (8/44, 18%). The majority of infections occurred in spring and summer, with higher incidence of S. aureus in summer months. Of S. aureus isolates, 89% (71/80) were found resistant to penicillin and 35% (28/80) to methicillin (MRSA). Of MRSA strains there was a high prevalence (85%) of the resistant phenotype penicillin/oxacillin/ fusidic acid/kanamycin/tetracycline, which commonly characterises the community-acquired strains (CA-MRSA). The resistance to macrolides among S. aureus isolates was 16.25% [I-phenotype 5% (4/80), MLSB (inducible resistance) 8.75% (7/80) and MLSB (constitutive resistance) 2.5% (2/80)]. Of S. pyogenes isolates, 23% were resistant to macrolides [MLSBl 18% (7/39) eae MLSBc 5% (2/39)].

**Conclusions:** Hand infections, especially at preschool age, are often monomicrobial and are usually caused by common flora of the skin and mouth such as S. aureus and S. pyogenes.

**Objective:** The aim of the study was to estimate incidence of HBoV infections in Slovenian children.

**Methods:** We tested respiratory samples of paediatric patients hospitalised in University Children’s hospital in Ljubljana with respiratory tract infection from July 2007 to June 2008. From total of 820 respiratory samples collected from 688 patients, there was 76.3% nasopharyngeal swabs, 19.5% throat swabs and 4.2% of other samples.

**Results:** In the first phase, human Bocavirus RNA was detected in 5.3% of samples. The second phase was characterised by headache in 96%, high fever in 99%, vomiting in 55% and meningeal signs in 90% of children. Meningitis (74%) dominated over meningoencephalitis (14%).
No difference in clinical parameters between children with meningitis compared to meningoencephalitis was found. Disturbances of consciousness (p<0.0001), seizures (p=0.03) and parietal involvement (p=0.01) were the only exceptions. Inflammatory changes in CSF were found in 89% of children. 81% of children had elevated cell count for polymorphonuclears and for lymphocytes. Abnormal protein concentration in the CSF (>0.45 g/l) was detected in 55/94 (59%) of children. IgM antibodies against TBEV were found early in the second phase in 89% of children.

Conclusions: Severe sequelae persist in two children (2%) while in three children (3%) the sequelae were classified as mild or moderate. Acquired aphasia, lasting tremor of the upper extremities, language deterioration, inversion of sleep and wakefulness, abnormal hyperkinetic movements and vertigo were found as permanent, but not progressing sequelae.

There is known relationship between the sequelae of TBE and the length of serological positivity of IgM antibodies. Immunological status in children with sequelae and those with prolonged duration of IgM seropositivity did not show any deficiency or autoimmune antibodies.

**P1958** Vertical transmission of CMV in HIV seropositive mothers at a Bangkok, Thailand hospital

P Bhattarakosol*, P Prisuwanna (Bangkok, TH)

**Objectives:** Cytomegalovirus (CMV), one of the opportunistic infections in HIV patients is known to cause a congenital infection. Vertical transmission of CMV can happen via placenta or in utero or perinatal transmission. The virus can infect several types of tissue and organs therefore variety of diseases with different symptoms can occur, varying from asymptomatic to severe leading to death. Transmission rate of CMV may be high up to 20–40% in primary infected mothers whereas only 0.2–2.2% has been reported in mothers with recurrent rate of CMV may be high up to 20–40% in primary infected mothers whereas only 0.2–2.2% has been reported in mothers with recurrent infection. Increase vertical transmission rate was previously reported in HIV infected mothers and it is believed that CMV may play role in the outcome of the diseases developed in newborns. Therefore, the prevalence of CMV infection of the HIV seropositive pregnant women and vertical transmission of CMV in newborns were investigated.

**Methods:** 43 HIV seropositive mothers who delivered at King Chulalongkorn Memorial Hospital, Bangkok, Thailand during January 2005 to June 2006 were recruited. EDTA blood and clotted blood were obtained from mother and newborn on the day of birth or within 72 hours after delivery. Urine from newborn was collected during the same period of time. The sera were determined for the presence of anti-CMV IgM and IgG by ELISA method. Plasma and white blood cells (WBC) were separated from EDTA blood. All clinical samples were extracted for viral DNA and detected for CMV-DNA by PCR.

**Results:** The prevalence of CMV infection in HIV infected mothers was 97.67% (42/43). Neither mothers nor newborns had anti-CMV IgM. CMV-DNA was detected in 17 (39.53%) newborn’s samples, i.e., 53% (9/17) in WBC; 47% (8/17) in plasma and 59% (10/17) in urine. Three cases (17.65%) were found CMV-DNA in all 3 samples, 4 cases (23.52%) were found in WBC and urine and the rest (58.82%, 10/17) was found in one of the 3 samples. Only 12 out of 17 mothers were able to detect CMV-DNA, 2 in plasma, 2 in plasma and WBC and 8 in WBC.

**Conclusion:** Almost all Thai HIV seropositive mothers in this study had already been infected with CMV. Urine is the best specimen for detection of CMV-DNA in newborn. The vertical transmission of CMV was shown at least 17.65% and possible up to 58.82%. No clinical symptoms was observed in all 17 newborns suggesting that they were asymptomatic CMV infection.

**P1959** Epidemiological and clinical aspects of measles cases hospitalised in a Bucharest infectious diseases clinic in 2005, during the measles outbreak in Romania (2005–2006)

R Botgrox*, S Florescu, A.M. Nicolescu, C.P. Popescu, P.I. Calistrut, E. Ceausu (Bucharest, RO)

**Introduction:** Measles is a disease affecting mostly children and possibly causing serious complications, mainly in the malnourished and immune compromised. It is the deadliest childhood rash illnesses, despite of being vaccine preventable. Starting with April 2005 an increase in the number of probable measles cases presenting to our hospital ward was observed. The hospitalisation of measles cases is mandatory in Roumania, and all presented patients were admitted in the hospital.

**Objectives:** Description of the basic epidemiological and clinical features of the measles patients admitted during 2005 in the “Dr. Victor Babes” Hospital Bucharest.

**Methods:** Retrospective clinical study of 444 measles patients admitted in our hospital between January and December 2005. Diagnosis of measles was established according to the CDC measles case definition. Laboratory confirmation was made by measuring the IgM antibodies against measles in the patient sera. Viral cultures were not performed. Rubella antibodies were measured at the same time.

**Results:** A total of 444 measles cases were admitted (9.7% of all 4601 hospitalised in Roumania in 2005), both children and adults. No imported case occurred. Fig.1 shows the cases number by month of onset. The hospitalisation period varied between 1 and 33 days (mean 7.4 days). Almost 90% of cases were under 15 years old and 16.4% were under 1 year of age. Information on vaccination status was provided in 39.6% of the cases, with only 9% of the cases having been vaccinated with at least one dose. The most frequent complications were measles pneumonia (57.9% over all, of which 22.6% in the 0–1 years and 27.2% in the 1–2 years age group), followed by rhinoadenoiditis and conjunctivitis (5.4% each). 14.2% of all patients were malnourished or immune deficient. 50 cases were diagnosed as nosocomial infections (epidemiological linkage proven) with additional 17 patients with possible nosocomial measles accounting for 15% of all patients. No deaths were observed.

Figure 1. Measles occurrence by month of admission.

**Conclusions:**
1. Despite the isolation measures, 15% of our patients developed nosocomial measles, probably due to the very high contagiousity index, combined with the large number of receptive individuals.
2. Data from our patients suggest a possible too low coverage of vaccination despite extensive vaccination campaigns in the last 15 years.
3. The most frequent complication was measles pneumonia which affected mostly the 0–2 years age group.
4. Mortality in our patient group was 0%.
Fungal epidemiology

**P1960** Aetiology of fever in children from urban and rural Tanzania


**Objectives:** Several studies have looked at the proportion of either malaria, pneumonia, diarrhoea or bacteraemia among fever cases in Africa but none of them has looked at the overall spectrum of aetiologies. We aimed at investigating the precise cause of fever episodes in children attending an outpatient clinic in urban (Dar es Salaam) and rural settings (Ifakara) in Tanzania.

**Methods:** All consenting children aged 2 months-10 years with an axillary temperature >38°C were recruited, except for those that required immediate supportive treatment. A detailed medical history and clinical examination were done to identify obvious foci of infection. A blood sample was taken to perform rapid tests for malaria and typhoid, blood culture as well as serological and molecular analyses. All had a throat and nasal swab taken for molecular investigation of respiratory pathogens. Urine was taken when no obvious cause of fever was found on clinical examination and a stool sample when diarrhoea was present. A chest X-ray was performed when IMCI criteria for clinical pneumonia were met. Each diagnosis was assigned a probability level (high, moderate, low) on the basis of pre-defined criteria.

**Results:** 1010 children were recruited, 510 in DSM and 500 in Ifakara. Preliminary results (prior to any molecular analysis or serologies) on the causes of fever (of high probability only) were as follows: 43% had acute respiratory infection (ARI) (30% URTI, 13% LRTI (6% clinical pneumonia and 7% pneumonia confirmed by X-ray)), 12% malaria, 9% diarrhoea (3% rotavirus and 6% bacterial or unknown), 8% urine infection, 4% typhoid, 2% skin infection, 1% occult bacteraemia and 21% still unknown at this stage. 8% had more than one diagnosis (high probability).

**Conclusion:** These results provide for the first time an accurate picture of the respective causes of fever in African children. As expected, ARI contribute to the largest burden of disease, most of them being URTI. There was a sizeable proportion of fevers due to typhoid documented within 3 months from the first diagnostics sample in the same person.

**P1962** The pattern of candidaemia in a tertiary referral hospital in the United Kingdom

I. Das*, P. Bunna, P. Nightingale, M. Patel (Birmingham, UK)

**Objectives:** Despite advances in diagnostic technology and antifungal agents, candidaemia continues to be associated with a high mortality. We analysed the epidemiology of candidaemia in our institution with an aim to optimise the management of this infection.

**Methods:** A prospective observational study of candidaemia over 33 months: 1 October 2005 to 30 June 2008.

**Results:** 107 episodes of candidaemia were detected in 102 patients. The incidence of candidaemia was 10.9 episodes/100,000 bed-days. 58% of episodes were hospital acquired and 51% of episodes were from intensive care units (ICU). Non-C. albicans species comprised 43% of the episodes. Overall, C. albicans was the commonest species accounting for 43% of episodes. The next commonest species were C. glabrata and C. parapsilosis accounting for 31% and 20% of episodes respectively. C. tropicalis, C. krusei, C. norvegensis and C. lusitaniae together comprised 7% episodes. During the first 15 months of the study period in 2005–2006, C. glabrata was the commonest species isolated. No resistance to amphotericin, fluconazole, voriconazole or caspofungin was detected in the C. albicans isolates. Resistance to amphotericin was not detected in any of the Candida species. Reduced susceptibility to fluconazole was detected in 30% of C. glabrata isolates. The most frequently identified focus of infection was the intravascular device (IVD) followed by a respiratory focus (35% and 21% of episodes respectively). A respiratory focus of candidaemia was associated with a higher mortality compared with IVD or an unidentifiable focus (53% vs 27% and 37% respectively). Delay in the initiation of antifungal therapy for >24 hours after a positive blood culture report occurred in 19% episodes. Four-week mortality was 37%. Multivariable analysis revealed association of advanced age and septic shock with mortality (P = 0.003 and 0.038 respectively).

**Conclusion:** Candidaemia remains an important cause of nosocomial infection with a high mortality. We report a higher proportion of non-C. albicans species, especially C. glabrata than that reported from other UK studies. This study highlights that the pattern of Candida species isolated from candidaemia is not always predictable from national studies and emphasizes the need for local surveillance. Effective measures against candidaemia should be considered in the empirical management of hospital acquired sepsis especially in ICU patients and the elderly.

**P1961** Candidaemia in Finland, 1995–1999 versus 2004–2007

E. Poikonen*, O. Lyytikäinen, P. Ruuta (Vantaa, Helsinki, FI)

**Objectives:** We studied the epidemiology of candidaemia in Finland (population, 5.3 million) by assessing the incidence and outcome as well as causative Candida sp. from 2004-2007 and compared the results to our previous study from years 1995–1999.

**Methods:** Since 1995 all Finnish clinical microbiology laboratories have notified all isolations positive for Candida sp. from blood to the National Infectious Diseases Register. Data collected include date of isolation, date of birth, sex, type of specimen, and place of treatment; since 2004, dates of death have been available from the Population Information System. A case of candidaemia was defined as a patient with at least one blood culture positive for Candida sp. Notifications of the same Candida sp. within 3 months from the first diagnostic sample in the same person were defined as one case.

**Results:** During 2004-2007, a total of 603 cases of candidaemia were identified. Median age of case-patients was 64 years (range 0–94 years) and 56% were male. The average annual incidence rate was 2.86 per 100,000 population (range by year 2.59–3.09). The rate was higher in males than in females (3.27 vs. 2.47), especially among patients aged <1 and >65 years. The highest rate was observed in males >65 years (12.23), and lowest in patients aged 1–15 years (0.25). The most frequent causative species was C. albicans (67%); C. glabrata ranked the second (19%), followed by C. parapsilosis (5%), C. krusei (3%) and C. tropicalis. The one-month case fatality varied between 33–38%.

Compared to years 1995–1999, the average annual incidence rate increased from 1.9 (range by year 1.7–2.2) to 2.86 during 2004–2007. According to sex the rate was higher in males than in females during both periods of observation. During 1995–1999, the highest rate were in males aged <1 year (11.9) and >65 years (7.4), although the increase in incidence occurred in males aged 16–65 years (from 1.0 to 2.4). C. albicans remained the common causative species (70–67%), but the proportion of C. glabrata increased from 9% during 1995–1999 to 19% during 2004–2007, and the proportion of C. krusei diminished from 8% to 5%, respectively. As a whole the proportion of non-albicans spp. was stable.

**Conclusion:** The incidence of candidaemia increased in Finland compared to 1990 s. The increase in incidence was associated with males aged >65 years. The proportion of C. glabrata rose, in spite of no shift towards non-albicans species. Crude mortality remained high.

**P1963** Prevalence of Candida metapsilosis and Candida orthopsilosis isolates in a Spanish yeast stock collection

I. Miranda-Zapico, E. Eruzo*, C. Marcos-Arias, J.L. Hernández Almaraz, A.J. Carrillo Muñoz, G. Quindós (Bilbao, Barakaldo, Barcelona, ES)

**Objectives:** To study the prevalence and antifungal susceptibility of Candida metapsilosis and Candida orthopsilosis among clinical isolates previously identified as Candida parapsilosis.
Methods: One hundred and twenty-one recent clinical isolates from our stock collection yielded during the last years were studied. The isolates included 72 from blood, 22 from genitalia, 19 from mouth and 8 from different clinical specimens. C. parapsilosis ATCC 22019 and ATCC 90018, C. metapsilosis ATCC 96143 and ATCC 96144 and C. orthopsilosis ATCC 96139 and ATCC 96141, were included as reference strains. Isolates were identified as C. parapsilosis by conventional mycological methods. These isolates were differentiated by a two-step DNA-based identification test and AFLP described by Tavanti et al. (Candida orthopsilosis and Candida metapsilosis spp. nov. to replace Candida parapsilosis groups II and III. J Clin Microbiol. 2005; 43: 284–292). Briefly, a 716-bp fragment of the SADH (secondary alcohol dehydrogenase) gene was amplified, purified, and digested with BanI. C. parapsilosis, C. metapsilosis, and C. orthopsilosis SADH amplicons contained, respectively, one, three, and zero BanI restriction sites.

Results: One hundred and sixteen isolates were C. parapsilosis sensu lato (95.9%), 3 C. metapsilosis (2.5%) and 2 C. orthopsilosis (1.6%). One isolate each of C. parapsilosis were from blood, genitalia and faeces. C. orthopsilosis was isolated from blood and genitalia. The antifungal susceptibilities to amphotericin B, anidulafungin, fluconazole, micafungin, and voriconazole of both blood isolates of C. metapsilosis and C. orthopsilosis and of 28 randomly-chosen blood isolates were tested by the CLSI M27A3 method. These isolates showed the same antifungal susceptibility patterns than C. parapsilosis blood isolates with a non-significant decrease of anidulafungin and micafungin MICs and with a non-significant increase of fluconazole MICs.

Conclusion: C. metapsilosis and C. orthopsilosis were identified as the cause of 2.8% of the invasive candidiasis previously attributed to C. parapsilosis. C. metapsilosis and C. orthopsilosis were also implicated in cases of superficial candidiasis.

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P1964 Do candiduria predict candidaemia in intensive care units in a Danish tertiary-care hospital?

J.K. Møller* (Aarhus, DK)

Objectives: Invasive mycoses are life-threatening opportunistic infections and have emerged as a major cause of morbidity and mortality in critically ill patients. This study aimed to determine whether candiduria is associated with the occurrence of nosocomial candidaemia and may serve as an early marker of disseminated infection.

Methods: A retrospective observational study. Microbiological data on patients in 4 ICUs at Aarhus University Hospital (AUH) during 1992–2008 were compiled from the laboratory information system MADS. AUH is a 1200 bed tertiary care hospital. All patients with at least one blood and/or one urine culture positive for Candida sp. were included.

Results: During the study period from 1992 to 2008, 306 patients had candidaemia and 892 had candiduria. The 30-days crude mortality rate for candidaemia at the AUH ICUs was about 40% during the period 2000–2005. Candida spp. became the most common cause of bloodstream infections in the ICUs at the end of the study period, less than 2% in 1992 and 21% in 2008. Candida albicans was by far the predominant species, causing about two-thirds of all cases of candidaemia (70%) and candiduria (68%). Other frequently isolated candida species in blood were C. glabrata (15%) and C. krusei (2%). Among the 306 patients with candidaemia, 124 (40%) had also candiduria but 57 of the 124 patients were cultured after the candidaemia was detected, 146 (48%) had their urine cultured but were culture-negative with candida spp., and 36 (12%) had not been examined for candiduria. Among the 892 patients with candiduria, 67 (7%) had subsequently a candidaemia. Thus on one hand, candiduria served as an early marker of candidaemia in 25% (67/270) of the cases. On the other hand, 92% (825/892) of patients with candiduria had not a candidaemia detected subsequently.

Conclusions: The data indicated that candiduria is not a reliable predictor of candidaemia. Furthermore, the urinary tract was probably the source for the candidaemia in less than half of the cases observed. Continued efforts to find better predictors of candidaemia are needed in order to identify patients at risk and to develop empirical treatment protocols to reduce the current high incidence and mortality of candidaemia in ICUs.

P1965 A most unusual case of Candida parapsilosis endocarditis in intravenous drug user

A. Galeri, M. Przybylo*, R. More, F. Sagliani, M. Walsh, R. Palmer (Blackpool, UK)

Background: Candida endocarditis is one of the most serious manifestations of candidiasis with mortality in excess of 50% without surgery and 41% with combined therapy and surgery. We present here an unusual case of Candida parapsilosis [CP] endocarditis in an intravenous drug user [IVDU] who miraculously survived/improved over 9-months with only intermittent antifungal treatment during three admissions/self discharge cycles before receiving surgical treatment.

Case study: A 47-year-old IVDU male underwent treatment for C. parapsilosis and mitral valve endocarditis. He intermittently admitted and self discharged on 4-occasions [stay 22d; 5d; 24d and 83 days] between Nov 06−07. He was first admitted with a history of headache, confusion and fever. CT brain revealed temporal lobe infarct and echocardiogram was consistent with moderate to severe aortic regurgitation [AR]. Mitral valve [MV] changes. His cerebrovascular event delayed his surgery during 1st admission. C. parapsilosis [CP] was isolated from total 10 sets of blood cultures [Nov 06−Sept 07] during the 4 admissions. Patient was partially treated during these hospital admissions with amphotericin, fluconosine, fluconazole and caspofungin. He refused to accept any treatment post self discharge and continued IVDU with amphetamines. After worsening of his ECHO findings during first 3 admissions; he presented in July 2007 [admission 4] with chest pain; minor cardiac vegetation and improved findings on ECHO; white cells 7; CRP 7 and then underwent a mitral and aortic valve replacement in Sept 2007. He was prescribed prolonged fluconazole at discharge post surgery.

Figure 1. TOE 21/11/06 confirming vegetation affecting 2 leaflets of the aortic valve associated with severe aortic regurgitation. Some oedema of the aortic valve annulus was noted with suspicion of early abscess formation. 1 cm vegetation noted.

Discussion: IVDUs are at an increased risk for infections through the unsterilised injection technique. fungal endocarditis is often difficult to diagnose because the presentation may be nonspecific and the disease typically occurs in otherwise critically ill patients with confusing clinical
pictures. The mortality rate remains 75–90% because of difficulty in making the diagnosis, lack of effective antifungal antibiotics, the need for surgical intervention in most cases. There is currently no consensus on the treatment of invasive Candida parapsilosis endocarditis. The therapeutic approach typically includes heart valve replacement surgery and administration of systemic anti fungal. Amphotericin B has been the most frequently used anti fungal. Fluconazole is an alternative. Detailed clinical findings and literature review to be presented.

**Figure 2.** 30/7/07 Vegetation hardly noticeable, possible prolapse of left coronary cusp. Severe regurgitation.

**P1966** Cryptococcal meningitis: 12-year experience in a single tertiary-care centre


**Objectives:** Cryptococcal infections are frequent in HIV-infected patients and are regularity looked after. This infection may occur in others immunosuppressives situations and, in these cases, diagnosis is often delayed. There are few reports on cryptococcal meningitis in non-HIV-infected patients in Korea. We reviewed the clinical features and efficacy of antifungal therapy in 33 patients who treated in a single tertiary care centre of Korea.

**Methods:** The medical records of 33 consecutive patients who had been admitted to our institution for cryptococcal meningitis from 1995 to 2007 were reviewed retrospectively. Cryptococcal meningitis was confirmed by positive culture of cerebrospinal fluid (CSF) or compatible clinical features plus a positive cryptococcal antigen test of CSF.

**Results:** 33 patients were analyzed and 30 cases were non-HIV patients. The outcomes were: complete cure in 23 cases, cure with sequelae in 4 cases, and mortality by treatment failure in 6 cases. The main initial manifestations were headache (84.8%), fever (54.5%) and seizure (33.3%). There was no statistical difference between patients who received amphotericin B treatment and those received fluconazole treatment mainly for long term, in terms of mortality rate. Factors significantly associated with unfavourable outcomes, including mortality and cure with sequelae cases, were fever, mental change, hearing impairment, initial high opening pressure of CSF (>250 mmH2O), and low initial absolute neutrophil count. On multivariate analysis, fever was independent predictors of unfavourable outcomes (odds ratio 17.3; 95% confidence interval, 1.0–28.3; \( P = 0.045 \)).

**Conclusions:** On the basis of our findings, it seems mandatory to closely observe cryptococcal meningitis patients with factors mentioned above.
started and subsequently potentiated with quinolones and linezolid. After 5 months of hospitalisation, thanks to an extensive rehabilitation program, a slow ameliorment of clinical-neurological picture allowed discharge, while all repeated testing for both cryptococcosis and tuberculosis became negative, and CD4+ count rose to 399 cells/µL.

**Discussion:** One single case report of a concomitant cerebral criptococcosis-tuberculosis was described in an AIDS patient from South Africa [Silber E, Neurology 1998;51:1213]. The present report, which exceptionally included an apparently concurrent, severe cryptococcal and tubercular meningoencephalitis in absence of evident underlying immunodeficiencies, represents a warning again underestimated of combined, rare infectious illnesses. From a pathogenetic point of view, an initial, slowly progressing meningeal tuberculosis (although diagnosed later in the disease course), probably prompted some grade of immunodeficiency, thus supporting the occurrence of brain cryptococcosis.

**P1969** 12-year post-mortem analysis of invasive mould infections in intensive care units in a tertiary care hospital

_C. Glaeser,* M. Schneemann, C. Ruf, A. Imhof (Zurich, CH)

**Background:** Invasive mould infections (IMI) are an important and increasing cause of morbidity and mortality in patients hospitalised on intensive care units (ICU).

**Methods:** All autopsy protocols of our university hospital performed during a 12-year period (1997–2008) were reviewed to investigate the incidence of IMI in ICU patients.

**Results:** 4251 protocols were analysed. 63 cases of mould infections were yielded in ICU patients. The median age was 63 years (range: 24–81) and there were 36 males and 27 females. The median ICU stay was 10 days (1–53). 59 cases with IMI and 4 patients with aspergilloma were diagnosed. The most frequent isolates were *Aspergillus* sp. (94%) followed by *Fusarium* sp. (2%), *Scedosporium* sp. (2%) and *Zygomycetes* (2%).

Fungal aetiology was diagnosed pre-mortem in only 28 (44%) of the patients. All these patients were treated with mould-active antifungal agents. However, in 40 (66%) patients, IMI was the primary cause of death. The major underlying conditions were haemato-oncological tumours (33%), followed by patients after solid organ transplantation (18%), patients with prolonged ICU stays after surgery (15%), patients with rheumatologic or immunological disorders receiving high dose corticosteroids (15%), patients with sepsis or cardiogenic shock (11%), and chronic lung diseases (4%), HIV patients (2%) and patients with solid organ cancer (2%).

**Conclusion:** This post-mortem analysis found IMI to be a frequent cause of death in ICU patients with a wide range of underlying conditions. The high incidence of not clinically entertained IMI confirms the importance of autopsy as a tool for quality control in medical diagnostic and therapeutic activity.

**P1970** Fungiscope: a global database for rare fungal infections

_M. Rüping, J.J. Veberescheld, C. Beisel, G. Fischer, C. Müller, A.J. Kindo, C. Lass-Flörl, W.J. Heinz, O.D. Cornely,* (Cologne, Aachen, Wurzburg, DE; Porur, IN; Innsbruck, AT)

**Objectives:** The incidence and clinical relevance of rare invasive fungal infections is increasing worldwide, but reliable information on their clinical course, diagnosis and treatment is scarce. To determine the clinical pattern of disease, to describe and improve diagnostic procedures, and therapeutic regimens, as well as to facilitate the exchange of clinical isolates, we are coordinating a global registry for rare invasive fungal infections.

**Methods:** Patients with cultural, histopathological, antigen, or molecular biologic evidence of invasive fungal infection may be included into the study. Those with infections due to *Aspergillus* spp., *Candida* spp., *Cryptococcus neoformans*, *Pneumocystis jiroveci* or any endemic fungal infection, such as coccidioidomycosis or histoplasmosis, as well as colonisation or other non-invasive infections are excluded. Data entry is accomplished via a web-based electronic case report form.

**Results:** By now, 65 patients with rare invasive fungal infections from a wide variety of pathologies have been included. The most common underlying conditions were chemotherapy for a haematologic malignancy (13%, n=15), haematopoietic stem cell transplantation (11%, n=13), Diabetes mellitus (13%, n=11) and/or stay at an intensive care unit (11%, n=13). The lungs were the most common site of infection (38%, n=26), followed by soft tissues (16%, n=11) and the parasanal sinuses (13%, n=3). 20 patients displayed disseminated disease. At the latest assessment, complete response to antifungal therapy was observed in 44% (n=28). The crude mortality rate was 39% (n=26). 5 patients (8%) were lost to follow up and in 2 patients (3%), final evaluation of clinical evolution is still pending.

**Conclusion:** The clinical relevance of rare invasive fungal infections is increasing steadily. In a short period of time, current cases from Europe, Asia and South America could be documented. Further investigators and coordinators are cordially invited to contribute to Fungiscope.

**P1971** A retrospective, multi-centre study of 25 cases of proven zygomycosis: risk factors associated with mortality


**Objectives:** Identify risk factors associated with zygomycosis mortality

**Methods:** A retrospective multicentre study was designed in order to identify proven cases of zygomycosis diagnosed during 2006 and 1st semester 2007 in 17 hospitals in Spain. Updated EORTC criteria were applied.

**Results:** Twenty five patients (20 males) median age 46 (range 21–76) with proven zygomycosis were identified. Most important underlying conditions were: haematologic 13 (52%); diabetes 8 (32%); solid organ transplant 3 (12%); trauma 2 (8%); solid tumour 2 (8%); rheumatologic 1 (4%); in one case no underlying condition was identified. From the haematologic patients 11 (84.6%) had active disease and all were neutropenic (<500 mm<sup>3</sup>). Steroids were used during the month prior to diagnosis by 13 patients (52%) and other immunosuppressants in 52%. Median days from hospital admission to diagnosis was 15 days. Zygomycosis was diagnosed in 11 cases (44%) by histopathologic or cytopathologic techniques in 7 cases (28%) by culture and in the remaining cases (28%) using both techniques. The infection was only rhinoorbitocerebral in 7 cases (28%) disseminated, affecting soft tissues and skin in 5 patients (20%) each, gastro-intestinal in 3 cases (12%), rhinoorbitocerebral+sinus in 2 cases (8.0%) and others in 3 (12%) cases. Ten patients (40%) received prophylaxis (7 fluconazole, 3 itraconazole). Eleven (44%) received empiric or pre-emptive treatment. Only 4/11 received liposomal amphotericin B, one combined with caspofungin. One received another lipid formulation and the remaining 6 patients received different treatment strategies. One patient did not receive any treatment, and the remaining 13 patients (52%) only started treatment after diagnosis. Seventeen patients (68%) had surgery. Overall mortality was 72% and attributable to zygomycosis in 13 cases (52%). 7/11 patients (53.8%) who received only anti-fungals after diagnosis died.

In a univariate analysis neutropenia and haemato-oncologic conditions were significantly associated with mortality, in multivariate analysis no risk factors were identified

**Conclusions:** Zygomycosis is a difficult fungal infection to diagnose with high mortality rate. Effective treatment that covers zygomycetes should be initiated early in order to reduce mortality. Neutropenia and haemato-oncologic conditions are risk factors, although in the multivariate analysis no risk factors associated with mortality were identified; this may have been due to the small sample size.
Filamentous fungi in cystic fibrosis: occult Scedosporium colonisation detected by selective media


Objectives: Scedosporium spp. are the second most frequent filamentous fungi after Aspergillus spp. isolated in cystic fibrosis (CF). Invasive infection is rare in patients prior to transplantation but fungal colonisation may be a risk factor for invasive disease, with its attendant high mortality, post lung transplant. As colonisation with more than one fungus is frequent, the prevalence of non-Aspergillus moulds may be underestimated in patients with CF. Furthermore, following recent taxonomic changes, contemporary epidemiological and microbiological data for Scedosporium colonisation in CF are required.

Methods: Expectorated sputum samples were collected from outpatients with CF from April to December 2008 and cultured on non-selective (Sabouraud’s agar + chloramphenicol/gentamicin), Mycosel and Scedosporium selective media (SceSel+) at 30ºC for up to 28 days. Colonies suspicious for filamentous fungi were identified to species level by routine laboratory methods. Scedosporium spp. were further characterised by RFLP analysis of the ITS region.

Results: Samples (n=152) were received from 52 patients (median age 24 years, IQ range 21–28 years, 41.1% male). The median number of samples per patient was 3 (range 1–6). Filamentous fungi were detected in 38 patients (67.9%). The most frequent pathogen was Aspergillus fumigatus (31 patients; 55.4%) followed by Scedosporium spp. (9 patients; 16.1%), Penicillium spp. (8 patients, 14.3%) and A. falcus (5 patients, 8.9%). ITS_RFLP analysis demonstrated that S. aurantiacum was the most frequent isolate (n=4 patients) followed by S. prolificans (n=3) and S. apiospermum (n=1); one isolate was speciated as P boydii species complex. Scedosporium spp. and A. falcus were more frequently present in mixed cultures compared with A. fumigatus (p=0.036 and 0.009 respectively). SceSel+ media was 44% more sensitive than non-selective media (88.8% vs. 44.4%). Up to 14 days of incubation was required to identify Scedosporium spp. in all samples.

Conclusions: The prevalence of Scedosporium colonisation in patients with CF in Sydney is 16.1%. S. aurantiacum is the predominant species. The use of selective media increases the rate of detection of Scedosporium spp. More than 50% of cases are missed with non-selective media due to overgrowth of other filamentous fungi.

Fungi from airway secretions of children with cystic fibrosis


Objectives: Last years there is an increasing trend of isolation of fungi from airway secretions of patients with cystic fibrosis (CF), which is accompanied with the presence of new fungal species. The aim of our study was to determine the prevalence as well as the species of fungi from airway secretions of children with CF.

Material and Methods: During a 23 months period (February 2007–December 2008) 2770 sputum or deep throat cultures were performed. The reason is that fungi participate in the inflammatory process and allergic bronchopulmonary reactions of these patients as well as that new pathogenic species are described, often resistant to many antifungal agents as Scedosporium apiospermum and Exophiala (Wangiella) dermatitidis.

Fungi from airway secretions of patients with CF

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>149</td>
<td>62.9</td>
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<tr>
<td>C. parapsilosis</td>
<td>17</td>
<td>7.2</td>
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<tr>
<td>C. lusitaniae</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>C. famata</td>
<td>3</td>
<td>1.3</td>
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<td>C. kefir</td>
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<td>0.4</td>
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<tr>
<td>A. fumigatus</td>
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<td>11.8</td>
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<tr>
<td>A. terreus</td>
<td>12</td>
<td>5.1</td>
</tr>
<tr>
<td>A. flavus</td>
<td>9</td>
<td>3.8</td>
</tr>
<tr>
<td>A. versicolor</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Scedosporium apiospermum</td>
<td>9</td>
<td>3.8</td>
</tr>
<tr>
<td>Exophiala (Wangiella) dermatitidis</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Geotrichon spp.</td>
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<td>0.4</td>
</tr>
<tr>
<td>Cryptococcus laurentis</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
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</table>

Conclusions: Suitable culture methods for the isolation and identification of fungi to species level is essential for patients with CF. The reason is that fungi participate in the inflammatory process and allergic bronchopulmonary reactions of these patients as well as that new pathogenic species are described, often resistant to many antifungal agents as Scedosporium apiospermum and Exophiala (Wangiella) dermatitidis.
Clinico-mycological profile of onychomycoses in patients attending dermatology clinics at a tertiary care centre in southern India

P Yegneswaran Prakash*, J Bai, G Shrijana (Manipal, IN)

Objectives: To study the pattern of clinical manifestation in onychomycoses patients and to identify the various dermatophytic, non dermatophytic mould and yeasts implicated in Onychomycoses.

Methods: The patient population involved those attending the dermatology outpatient clinic during the time frame of January 2006 to August 2008. During this period a total of 520 patients representing both genders who volunteered by their consent after being informed about the study with clinical features of Onychomycoses were recruited. Patients on prior anti fungal therapies for more than 4 weeks were excluded. Nail clippings collected were portioned and used for direct microscopy and recovery of the aetiological fungal agent by culture. Identification of the Filamentous/Yeast fungi was done based on the macroscopic and microscopic features and by performing physiological tests as per the standard mycological protocols.

Results: The most common type of clinical presentation was distal lateral subungual Onychomycoses and the common organism isolated from this type of presentation was Fusarium spp, followed by Total dystrophic Onychomycoses caused by Dermatophytes. The common age group that was affected by Onychomycoses was those in the 41 to 50 years age group with a mean age of 41.47 years. The type of occupation with the highest presentation of Onychomycoses were those involved with work that required them to be in constant contact with water like the housewives 26.2%, and the paddy field workers 23.8%. Diabetes mellitus was associated with Onychomycoses in 31.3% and with hypertension in 18.8% of the patients. A prior trauma to the nails was associated with Onychomycoses in 54.8% of the patients, and the p value of 0.04 proved this association to be significant. In some instance exotic agents of onychomycoses like Onychocolacanadensis, Lasiodiplodia theobromae were isolated.

Conclusion: The burden of Onychomycoses in the society, causing morbidity cannot be ignored. Although in a developing country like ours where there are more immediate and life threatening disease to combat, it is important to note that a great majority of Onychomycoses sufferers are unseen or hidden patients, and if they are to report to a dermatologist the actual number would be much higher. It is important to recognize, identify and confirm Onychomycoses due to non dermatophyte filamentous fungi as they may not be responsive to even newer antifungal agents.

Pharmacokinetics & pharmacodynamics of aminoglycosides & lipoglycopeptides

P1977 In vitro pharmacodynamic properties of colistin methanesulfonate and amikacin against Pseudomonas aeruginosa isolates from patients with cystic fibrosis

C. Bozkurt Güzeli*, A. Gercelker (Istanbul, TR)

Objectives: The in vitro pharmacodynamic properties of colistin methanesulfonate and amikacin were investigated by studying time-kill kinetics, and postantibiotic effect (PAE) against strains of Pseudomonas aeruginosa isolated from patients with cystic fibrosis.

Methods: Synergy was investigated at 0.5×, 1× and 5×MIC concentrations of antibiotics in vitro. Comparison of the PAE for each strain was done assuming a monocompartment open model with zero-order absorbtion (ev perfusion) and a first-order elimination. The Sawchuk-Zaske equation and the SPSS 11.5. statistical analysis program were used for the pharmacokinetic calculations.

Results: Synergy and additive effects were detected with colistin methanesulfonate and amikacin combination, at 1×MIC concentrations produced PAEs of 5.5h and 4.2h for P. aeruginosa and 4.7h and 5.3h for S. aureus. When the antibiotics were used in combination at a concentration of 20 times of the MIC values the PAEs were prolonged to a value of 4.8h.

Conclusion: Consequently, the findings of this study may play useful role in selecting the appropriate combinations when a single agent is inadequate, and may have important information for optimising the dose intervals. Optimisation of antibiotic dosing intervals will allow us for the design of rational dosage regimens, thereby reducing the costs and development of resistance and toxicity during therapy.

P1978 Pharmacokinetics of amikacin at a Portuguese burn unit

M. Caetano, M. João Rocha, L. Cabral*, C. Crazeiro, G. Tribuna, M.A. Cabral (Coimbra, PT)

Objectives: Burns can cause serious complications like electrolyte imbalance and infection. Amikacin is an antibiotic available to treat patients with suspected or documented severe Gram negative (−) infections. The aim of this study is to evaluate the administration and the response to amikacin in burn patients at a burn unit, and to analyse their pharmacokinetic behaviour.

Methods: This is a retrospective study conducted between January 2005 and December 2008. The study population included patients on amikacin treatment for Gram(−) infections. The pharmacokinetic study was done assuming a monocompartment open model with zero-order absorption (ev perfusion) and a first-order elimination. The Sawchuk-Zaske equation and the SPSS 11.5. statistical analysis program were used for the pharmacokinetic calculations.

Results: This study involved 76 patients with an average age of 48±18 years and an average weight of 72±15 kg. The mean Total Body Surface Area (TBSA) burned was 22±10% and the average length of treatment was 14±9 days. 47% of the patients had a burned TBSA>20%. The amikacin standard dose was 18±9 mg/kg/day. 300 amikacin plasmatic levels were collected. Out of the through/peak pairs, only nearly 15% were within the recommended range. The average analytic values found were, serum creatinine: 0.8±0.7 mg/dL, albumin: 3.3±0.6 mg/dL, potassium: 4.0±0.6 mg/dL. The C-Reactive Protein (PCR) values ranged from 1.8 to 39 mg/dL. The average pharmacokinetic parameters estimated were Volume of Distribution (VD): 0.5±0.8 L/kg, serum half-life (t1/2): 3.1±3.6h and Clearance (CL): 8±6.4 L/h for patients with TBSA<20%, and VD: 0.5±0.6 L/kg, t1/2:2.40±3.70h and CL: 8.50±13.90 L/h for patients with TBSA>20%. After the pharmacokinetic adjustment, amikacin doses had to rise to 34.0±20.0 mg/kg/day.

Onychomycosis in Tehran: mycological study of 504 patients

J Hashemi* (Tehran, IR)

Background: Onychomycosis, a common nail disorder results from the causative fungi of onychomycosis in the population in Tehran, Iran.

Objectives: The purpose of present study was to isolate and determine the causative fungi of onychomycosis in the population in Tehran, Iran.

Methods: Totally nail materials of 504 patients with prediagnosis of onychomycosis during 2005, were examined both with direct microscopy observation of fungal elements in KOH preparations and culture to identify the causative agent. All samples were inoculated on (1) Sabouraud dextrose agar (SDA, Merk) (2) SDA with 5% chloramphenicol and cycloheximide in duplicate for dermatophyte and (3) SDA with 5% chloramphenicol trimelate for mould isolation.

Results: Out of a total of 504 cases examined, 216 (42.8%), were out of the nail plate by a dermatophytes, yeasts or mould species that these fungi give rise to some diverse clinical presentations. These fungi were isolated from patients with cystic fibrosis (CF). The common type of clinical presentation was distal lateral subungual Onychomycoses and the common organism isolated from this type of presentation was Fusarium spp, followed by Total dystrophic Onychomycoses caused by Dermatophytes. The common age group that was affected by Onychomycoses was those in the 41 to 50 years age group with a mean age of 41.47 years. The type of occupation with the highest presentation of Onychomycoses were those involved with work that required them to be in constant contact with water like the housewives 26.2%, and the paddy field workers 23.8%. Diabetes mellitus was associated with Onychomycoses in 31.3% and with hypertension in 18.8% of the patients. A prior trauma to the nails was associated with Onychomycoses in 54.8% of the patients, and the p value of 0.04 proved this association to be significant. In some instance exotic agents of onychomycoses like Onychocolacanadensis, Lasiodiplodia theobromae were isolated.

Conclusion: The burden of Onychomycoses in the society, causing morbidity cannot be ignored. Although in a developing country like ours where there are more immediate and life threatening disease to combat, it is important to note that a great majority of Onychomycoses sufferers are unseen or hidden patients, and if they are to report to a dermatologist the actual number would be much higher. It is important to recognize, identify and confirm Onychomycoses due to non dermatophyte filamentous fungi as they may not be responsive to even newer antifungal agents.
Conclusions: As expected, these results show a great interindividual variation in amikacin pharmacokinetic parameters. Taking the trough/peak values into account, the standard dose used is not adequate for this group of patients. So, the routine pharmacokinetic adjustment of this antibiotic would be useful. Further studies would be important to find an ideal standard dose for these patients as well as the better frequency of administration of amikacin.

P1979 Quantitative comparison of aminoglycoside nephrotoxicity in rats for effective screening and evaluation of new derivatives, and dosing rationales that minimise toxicity


Objectives: Aminoglycosides (AG) are a well-known class of antibiotics with an established record of efficacy, but limited in their use because of the risks of oto- and nephrotoxicity. To support the development of the next generation of AGs with an improved antibacterial spectrum and clinical safety (neoglycosides), we have refined a rat toxicity model to effectively quantitate AG nephrotoxic potential. The model was based on integration of extensive past research on AG nephrotoxicity and allows for effective screening of novel AGs with potentially reduced nephrotoxicity.

Methods: Our standard rat nephrotoxicity study design used 14 days of once-daily dosing. We also monitored changes in serum markers of glomerular filtration rate (GFR) and microscopic examination of kidney slices using H&E staining, as well as scoring of cellular necrosis, tubular dilation, and basophilia. This rat model provided a consistent measure of AG nephrotoxicity, as evidenced by the reliable dose-response of serum creatinine changes for gentamicin across a number of independent studies (no change at 10 mg/kg, mild elevation at 30 mg/kg, and >2x elevation/mortality at 100 mg/kg).

Results: Neomycin, sisomicin, gentamicin, apramycin, tobramycin, paromomycin, and amikacin were evaluated. Their nephrotoxicity ranking in this rat model (minimum dose that affects GFR) correlates well with their relative clinical nephrotoxicity, where clinical data are available. AG induced kidney changes were detected by H&E at doses many multiples below those that cause a GFR functional deficit (30X for gentamicin). Consistent with prior work and the model that kidney uptake of AGs is a saturable process, once-daily dosing of gentamicin was significantly less toxic than twice- or three-times daily dosing of the same total daily dose. Also, supporting the model that AG nephrotoxicity is correlated to the total duration of treatment, we found that limiting the duration of dosing to 5 days allows for doubling the dose of gentamicin without a significant increase in toxicity compared with 14 days of dosing.

Conclusions: Our rat model allows for a consistent evaluation of the nephrotoxic potential of AGs, and also points to conditions that minimise toxicity. This model should allow for the reliable evaluation of the nephrotoxic potential of new AG derivatives, and guide their selection for further clinical development. This work also provides a rationale for shorter course dosing of AGs to minimise toxicity.

P1980 Assessment of amikacin plasma level in a referral hospital in southern Iran

S. Namazi*, M. Sagheb, G. Vessal, M. Hashempoor (Shiraz, IR)

Objective: Amikacin exhibits concentration-dependent effects, therefore plasma level monitoring of amikacin to improve treatment efficacy and safety is an important factor. In our hospital amikacin plasma level monitoring is not performed routinely. This study was designed to determine the appropriate use of amikacin in Namazi hospital in Iran.

Methods: All patients who received amikacin in internal wards of Namazi hospital were selected during a 1-year period. Trough and peak plasma levels were drawn from patients at steady state condition. Samples were assayed by a turbidometer autoanalyzer (Cobas-Mira, Roche, Germany). A log sheet that included 12 items regards indication, dosing, administration, and monitoring of amikacin was provided. This log sheet was completed for each patient and compared to a standard guideline designed by a clinical pharmacist. A score of 1 or 0 was given to each variable depending on the fact that each variable was evaluated appropriately or inappropriately, respectively. A total score was given to each patient by adding the scores for each variable. Statistical analysis was performed using SPSS version 11.5.

Results: 63 patients were enrolled into the study. The age range was 18–92 (55±22.5) years. The most common cause of admission was fever (63.49%). Pyelonephritis was the primary indication for amikacin therapy. 76% of patients had a creatinin clearance <50 ml/min based on Cockcroft-Gault formula. Only in 25% of patients amikacin dose was adjusted based on creatinin clearance. The averages trough and peak levels of amikacin were 15.67±7.79 mcg/ml and 15.67±7.79 mcg/ml, respectively. 45% and 38% of trough and peak levels respectively were in therapeutic range. Data analysis of log sheets indicated that the average score for appropriateness of amikacin usage was 5.8±0.3 (4–10) out of 12. Conclusion: Amikacin use and serum levels were inappropriate in most of our patients. Standard treatment guideline should be provided and implemented in order to improve amikacin use.

P1981 Aminoglycosides: acoustic toxicity screening

R. Morosi*, M. Georgescu, A. Pasca, A. Hristea, V. Arama, E. Kocaks, M. Rafa (Bucharest, Cluj-Napoca, RO)

Objectives: Aminoglycosides (AG) are widely prescribed despite their notorious toxicity. The aim of this study was to monitor and to characterise the acoustic toxicity of the AG using an otoacoustic emission (OAE) analyser. AG cause irreversible hearing loss, starting with high frequencies and progressing toward conversational frequencies (0.5–2 kHz), by destroying the cochlear cells. The integrity of these cells could be analyzed by recording the faint sounds that they produce – otoacoustic emissions.

Methods: We performed a prospective study of 49 patients receiving gentamicin (G) during 2007–2008. We used serial OAE recordings with an ILO 92 analyser (1–8 kHz) on at least 3 occasions: at the start, during the course and after the cessation of therapy (1–6 months). The recordings were performed at the patients’ bedside, independently of their status of consciousness. The method is fast, non-invasive, accurate and doesn’t require an ENT specialist. We included patients presenting OAEs. The exclusion criteria were acute middle ear affection or OAE absence on the initial test. Ototoxicity was defined (related to the highest previously recorded frequencies) as a decrease of at least 20 dB at one frequency, a 10 dB decrease at 2 adjacent frequencies or a loss of 3 adjacent frequencies.

Results: We studied 49 patients, 24/25 female/male, mean age 37.24 (3–70 years old), who received G for 4 to 42 days: 24 patients were treated for less than 10 days and 25 patients had longer courses of treatment. Hearing impairment was observed in 10 patients (20.4%) and appeared during the therapy or in the next 3 months, with the loss of one or 2 high frequencies that occurred unilaterally in 9 out of 10 cases. The acoustic damage correlates with the length of AG treatment (50% for long versus 8.3% for short courses). We didn’t find a statistical correlation between the degree of impairment and the age, doses or association of another ototoxic drug, although the hearing loss was slightly higher in those with concomitant exposure: 4 out of 10 versus 13 out of 39 non-exposed.

Conclusions: OAE monitoring of AG treatment is a very useful way for detecting and preventing acoustic toxicity, because it could warn about hearing loss before damage of the conversational frequencies. The accuracy is similar to the classical methods, but it is easier to perform and faster.
**[P1982]** Validation of a high-trough vancomycin nomogram to achieve trough concentrations of 15 to 20 mg/L


**Objective:** We previously validated a vancomycin (VAN) nomogram at Detroit Receiving Hospital to achieve targeted trough concentrations of 5–20 mg/L (Karam et al, Pharmacotherapy 1999). Recent guidelines (Rybak et al, AJHP 2009; 82–98) proposed by the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists recommend increasing the targeted trough serum concentration of VAN to 15–20 mg/L. The objective was to evaluate and validate the ability of a revised VAN dosing nomogram to achieve the targeted trough serum concentrations of 15–20 mg/L.

**Methods:** This was a prospective multi-centre validation study from 4 U.S. academic centres. The construction of the revised nomogram utilised actual body weight, estimated creatinine clearance (CrCl), and median CrCl 63.0 ml/min (37–105). 37.6% patients were in the intensive care unit at time of initial VAN, 79.8% were placed on VAN for empiric therapy. 75.2% received concomitant antmicrobial therapy. Most common dosage and interval for VAN was 1000 mg every 12 hours (20.2%), followed by 1250 mg every 12 hours (17.4%). 54.1% achieved targeted attainment (TA) of 15–20 mg/L, 66.4% achieved TA of 12–14 mg/L. 13.8% of patients had TA > 20 mg/L and 12.8% < 15 mg/L. Safety: 11/109 patients developed nephrotoxicity; however, 72.3% of these patients that experienced nephrotoxicity were on concomitant nephrotoxic agents (i.e. aminoglycosides, colistin, acyclovir).

**Conclusion:** This revised high-trough VAN nomogram reveals that the ability to achieve a small increment of 5 mg/L (15–20 mg/L) was somewhat variable. However, allowing for a range of 13–22 mg/L achieved approximately 75% of TA and would appear to be clinically acceptable for empiric dosage adjustment.

**[P1983]** Pharmacokinetics analysis of vancomycin initial dose in critically ill patients


**Objectives:** Pharmacokinetics analysis of vancomycin initial dose in critically ill patients.

**Methods:** Design: observational prospective study. Duration: six months (January 2008–July 2008). Inclusion criteria: all patients in a critical care unit and treated with vancomycin. Vancomycin initial dose: 1000 mg bid i.v. From the pharmacokinetic registry of pharmacy department standard VAN equations and population pharmacokineticstodetermine VAN dose and interval (Karam et al, Pharmacotherapy 1999; Ducharme et al, Ther Drug Monit 1994). Exclusion criteria consisted of patients with weight > 110 kg, CrCl < 30 ml/min or > 110 ml/min, and rapidly changing or unstable renal function or volume of distribution. All patients with empiric VAN dosage determined by the nomogram had serum concentrations measured between the 3rd and 5th doses according to standard pharmacy procedures.

**Results:** 109 eligible patients were identified. Baseline characteristics: Median age 56 yrs (range 18–86), median weight 69.0 kg (47–110), and median CrCl 63.0 ml/min (37–105). 37.6% patients were in the intensive care unit at time of initial VAN, 79.8% were placed on VAN for empiric therapy. 75.2% received concomitant antmicrobial therapy. Most common dosage and interval for VAN was 1000 mg every 12 hours (20.2%), followed by 1250 mg every 12 hours (17.4%). 54.1% achieved targeted attainment (TA) of 15–20 mg/L, 66.4% achieved TA of 12–14 mg/L, 13.8% of patients had TA > 20 mg/L and 12.8% < 15 mg/L. Safety: 11/109 patients developed nephrotoxicity; however, 72.3% of these patients that experienced nephrotoxicity were on concomitant nephrotoxic agents (i.e. aminoglycosides, colistin, acyclovir).

**Conclusion:** This revised high-trough VAN nomogram reveals that the ability to achieve a small increment of 5 mg/L (15–20 mg/L) was somewhat variable. However, allowing for a range of 13–22 mg/L achieved approximately 75% of TA and would appear to be clinically acceptable for empiric dosage adjustment.

**[P1984]** High-dose daptomycin for complicated Gram-positive infections


**Objective:** We previously validated a vancomycin (VAN) nomogram at Detroit Receiving Hospital to achieve targeted trough concentrations of 5–20 mg/L (Karam et al, Pharmacotherapy 1999). Recent guidelines (Rybak et al, AJHP 2009; 82–98) proposed by the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists recommend increasing the targeted trough serum concentration of VAN to 15–20 mg/L. The objective was to evaluate and validate the ability of a revised VAN dosing nomogram to achieve the targeted trough serum concentrations of 15–20 mg/L.

**Methods:** This was a prospective multi-centre validation study from 4 U.S. academic centres. The construction of the revised nomogram utilised actual body weight, estimated creatinine clearance (CrCl), standard VAN equations and population pharmacokinetics to determine VAN dose and interval (Karam et al, Pharmacotherapy 1999; Ducharme et al, Ther Drug Monit 1994). Exclusion criteria consisted of patients with weight > 110 kg, CrCl < 30 ml/min or > 110 ml/min, and rapidly changing or unstable renal function or volume of distribution. All patients with empiric VAN dosage determined by the nomogram had serum concentrations measured between the 3rd and 5th doses according to standard pharmacy procedures.

**Results:** 109 eligible patients were identified. Baseline characteristics: Median age 56 yrs (range 18–86), median weight 69.0 kg (47–110), and median CrCl 63.0 ml/min (37–105). 37.6% patients were in the intensive care unit at time of initial VAN, 79.8% were placed on VAN for empiric therapy. 75.2% received concomitant antmicrobial therapy. Most common dosage and interval for VAN was 1000 mg every 12 hours (20.2%), followed by 1250 mg every 12 hours (17.4%). 54.1% achieved targeted attainment (TA) of 15–20 mg/L, 66.4% achieved TA of 12–14 mg/L, 13.8% of patients had TA > 20 mg/L and 12.8% < 15 mg/L. Safety: 11/109 patients developed nephrotoxicity; however, 72.3% of these patients that experienced nephrotoxicity were on concomitant nephrotoxic agents (i.e. aminoglycosides, colistin, acyclovir).

**Conclusion:** This revised high-trough VAN nomogram reveals that the ability to achieve a small increment of 5 mg/L (15–20 mg/L) was somewhat variable. However, allowing for a range of 13–22 mg/L achieved approximately 75% of TA and would appear to be clinically acceptable for empiric dosage adjustment.

**Table 1. Initial susceptibility of pathogens**

<table>
<thead>
<tr>
<th>Organism</th>
<th>VAN (mg/L)</th>
<th>MIC (mg/L)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MIC50</td>
<td>MIC50</td>
</tr>
<tr>
<td></td>
<td>MIC90</td>
<td>MIC90</td>
</tr>
<tr>
<td>Enterococci (N = 36)</td>
<td>≥32</td>
<td>≥64</td>
</tr>
<tr>
<td>MRSA (N = 54)</td>
<td>2</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Conclusions: HD DAP is a viable treatment option in pts with complicated Gram-positive infections, and is associated with favourable clinical response and safety profile.

**P1985** Vancomycin and daptomycin susceptibility related to the accessory gene regulator locus in MRSA using in vitro pharmacokinetic/pharmacodynamic models

C. Vidalúa*, G.W. Kautz, S.M. Seo, M.J. Rybak (Detroit, US)

Objectives: The agr locus is a quorum-sensing gene cluster involved in the pathogenesis of *Staphylococcus aureus* (SA) that has been associated with vancomycin (VAN) treatment failure with a higher propensity for agr group II. This study evaluated the relationship between VAN and daptomycin (DAP) reduced susceptibility and agr group and function in methicillin-resistant SA (MRSA) after exposure to VAN using two different in-vitro PK/PD models.

Methods: Two isogenic pairs of agr+/agr− group I and II MRSA were exposed to VAN for 72 or 192h. Selected VAN dosing regimens simulating fAUC/MIC ratios from 7 to 224 (31–1000mg q 12h) were evaluated in duplicate in a one-compartment PK/PD model and 112 (500mg q 12h) in a two-compartment PK/PD model with simulated endocardial vegetations (SEV), agr function as well as resistance and tolerance to VAN and DAP were evaluated over 72 or 192 h. MICs were determined according to CLSI guidelines.

Results: Pre-exposure MICs were 0.5 and 1 for VAN and 0.25 mg/L for DAP for all test strains. In the one-compartment model, fAUC/MIC of 7 and 56 resulted in an increase in MIC of 6 and 3× for VAN and 4 and 2× for DAP for the agr I and II null strains versus 3 and 1× for VAN and 4 and 1× for DAP for the agr positive strains, respectively. A VAN fAUC/MIC of 224 was needed to suppress VAN and DAP resistance in the agr I and II null strains versus 112 and 56 in the agr I and II functional strains, respectively. In the SEV model, a VAN fAUC/MIC of 112 led to the emergence of VAN resistance at 144h for the agr II null strain versus 192h for the isogenic agr+ strain. Mutants of the agr null strain recovered from plates containing 3× the baseline VAN MIC at 192h exhibited increases in both VAN and DAP MICs of 4–6× and 6–8×, respectively. In contrast, mutants of the agr positive strain demonstrated increases in the VAN and DAP MIC of only 2×. No change in agr function was observed with any VAN regimen.

Conclusions: We have demonstrated a more rapid rate of emergence of VAN resistance in isogenic MRSA agr null compared to agr functional strains at human simulated VAN concentrations. The emergence of VAN resistance was also associated with an elevation in the DAP MIC. Further research is warranted to understand the relationship of agr function and SA susceptibility to VAN and DAP.

**P1986** The anti-staphylococcal activity of telavancin in comparison to teicoplanin studied in an in vitro pharmacokinetic model of infection

K. Bowker*, A. Noel, S. Tomaselli, A.P. MacGowan (Bristol, UK)

Objectives: Telavancin (tela) is a lipoglycopeptide antimicrobial with a double mechanism of action, acting on both the bacterial cell wall and membrane. It has a broad spectrum of in vitro activity against Gram positive pathogens. Clinical trials have shown tela to be non-inferior in effectiveness to vancomycin in treatment of skin infection and hospital acquired pneumonia. However, there is little pre clinical and no clinical data on the comparative activity of tela and teicoplanin (teico). In this study, we used an in vitro pharmacokinetic (PK) model to compare the antibacterial effects of tela and teico against 2 strains of MRSA and a single VISA strain.

Methods: A single chamber dilutional PK model was used to simulate free drug serum concentrations: tela Cmax 10 mg/L, 12 h concentration 3.3 mg/L, 24 h concentration 1.1 mg/L, teico Cmax 4.5 mg/L, 12 h concentration 2 mg/L, 24 h concentration 0.75 mg/L. Two strains of MRSA were used, tela MICs 0.19 and 0.25 mg/L, teico MICs 0.19 and 0.12 mg/L. The VISA strain tela MIC was 0.75 mg/L, teic MIC 16 mg/L.

Experiments were performed in at least triplicate at an initial inoculum of 10⁶ CFU/ml. Antibacterial effect was measured by log change in viable count at 12h (d12), 24h (d24), 36h (d36) and 48h (d48). The area-under-the-bacterial-kill curve was between 0–24h (AUBKC24) and 0–48h (AUBKC48).

Results: As the killing kinetics of both agents were the same for both MRSA strains, the data was combined and compared (table).

<table>
<thead>
<tr>
<th></th>
<th>MRSA (2 strains)</th>
<th>VISA (1 strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f12</td>
<td>f36</td>
</tr>
<tr>
<td></td>
<td>tela</td>
<td>telavancin</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Conclusion: Tela produced a greater anti staphylococcal effect on the MRSA strains than teico, showing more rapid early killing and lack of grow back. Tela was also significantly more effective against the VISA strain than teico. This data helps support the clinical use of tela as an alternative therapy to teico.

**P1987** Pharmacodynamic comparison of daptomycin and vancomycin against clinical isolates of methicillin-resistant *Staphylococcus aureus* in three European countries

A. Cauza*, A. Rodríguez-Gascón, E. Cercenado, C. Betriu, A. Labora, J.L. Pedraz (Vitoria, Madrid, ES)

Objective: A reduction in the efficacy of vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA) strains with high vancomycin MIC values (1–2 mg/L) has been described in recent studies. Accordingly, the objective of the current study was to calculate the probability of attaining targeted pharmacodynamic exposure for various regimens of vancomycin and daptomycin against clinical isolates of *S. aureus* collected in Belgium, United Kingdom and Spain.

Methods: Susceptibility data to vancomycin and daptomycin of clinical MRSA isolates from Belgium (n = 511), United Kingdom (n = 97) and Spain (n = 298) were analyzed. Steady-state exposure was assessed for the following antibiotic regimens: vancomycin (1000 mg/12h, 1000 mg/8h, 2000 mg/12h, 1500 mg/8h and 1500 mg/6h) and daptomycin (4, 6, 8 mg/kg every 24h). Mean pharmacokinetic parameters and their distribution were extrapolated from published patients’ studies for each antibiotic. The area under the concentration-time curve divided by the MIC (AUC/MIC) was utilised as the pharmacodynamic parameter to predict vancomycin and daptomycin efficacy (pharmacodynamic targets of >400 and >438, respectively). For vancomycin, Cmin/free/MIC was also calculated (values of >4). A 10000-patient Monte-Carlo simulation was performed to calculate the AUC/MIC for vancomycin and daptomycin and Cmin/free/MIC for vancomycin. Cumulative fraction of response (CFR) for the requisite pharmacodynamic target was calculated weighing the probability of target attainment at each MIC by the percentage of organism with that MIC.

Results: In Belgium, CFR higher than 90% was achieved with vancomycin doses higher than 1000 mg/8h. However, in United Kingdom and in Spain, CFR >90% is only achieved with the highest dose: 1500 mg/6h. The differences are due to differences in susceptibility of the isolates; whereas in Belgium 100% of the isolates presented MIC values ≤1 mg/L, only 64% and 69% of the isolates reached these MIC values in United Kingdom and in Spain, respectively. Concerning daptomycin, CFR values higher than 90% are achieved with the lowest dose (4 mg/Kg) for isolates from Belgium, United Kingdom and Spain. CFR values of 100% were achieved with 6 mg/Kg and 8 mg/Kg in the isolates of the three countries.

Conclusion: Daptomycin had a greater likelihood of obtaining its requisite pharmacodynamic exposure against MARSa due to the excellent activity against all strains recovered in Belgium, United Kingdom and Spain.
**[P1988]** In vitro bactericidal activity of daptomycin versus vancomycin Cmax concentrations in the presence of human albumin physiological concentrations against vancomycin-susceptible but tolerant methicillin-resistant *Staphylococcus aureus*

M. Torrico, L. Aguilar, N. Gonzalez, M.J. Gimenez, F. Cafini, L. Alou, D. Sevillano*, R. Cleeland, J. Prieto (Madrid, ES; East Hanover, US)

**Objective:** To study the antibacterial activity (log10 reduction) and time to obtain bactericidal activity (time to obtain ≥3 log10 − 99.9% − initial inocula reduction, T99.9%) in the presence of physiological concentrations of human albumin by concentrations similar to Cmax obtained in serum with 6 mg/kg od daptomycin and 1g bid vancomycin regimens, against vancomycin-susceptible but tolerant methicillin-resistant *Staphylococcus aureus* (MRSA).

**Methods:** Killing curves were performed with final inocula of approx. 10^7 cfu/ml, and a final concentration of 98.6 mg/ml daptomycin or 65.7 mg/ml vancomycin, using as media Mueller-Hinton broth (Cmax-MH) and MH broth with 4 g/dl human albumin (Cmax-HAlb). In the case of daptomycin, media were supplemented with Ca^2+ (by adding 100 mg/l of Ca^2+ in media containing albumin and 50 mg/l in media without albumin) in order to obtain a physiological free Ca^2+ concentration.

**Results:** MIC/MBC (mg/l) of daptomycin and vancomycin, T99.9% (h) and log10 initial inocula reduction at 12h/24h are shown in the Tables.

<table>
<thead>
<tr>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax-MH</td>
<td>2/5.8</td>
<td>5.9</td>
<td>1</td>
</tr>
<tr>
<td>Cmax-HAlb</td>
<td>3/5.9</td>
<td>6.0/0.0</td>
<td>2</td>
</tr>
</tbody>
</table>

**Conclusions:** Daptomycin Cmax exhibited rapid (≤3h) bactericidal activity while vancomycin did not achieve bactericidal activity along 24h against vancomycin-susceptible but tolerant MRSA. The presence of human albumin physiological concentrations slightly delayed T99.9% for daptomycin.

**[P1989]** Vancomycin protein binding in a general hospital population with *S. aureus* sepsis

A. Locero*, M. Darville, A.P. MacGowan (Bristol, UK)

**Objectives:** Pharmacodynamic assessment of antibacterials is now most often conducted using free drug concentrations. This means that protein binding (PB) is a vital pharmacokinetic measure and the distribution of PB values in patients needs to be known for Monte Carlo modelling. In man, vancomycin outcome has been predicted by total drug AUC/MIC ratio with little reference to PB. Although there are published PB data for vancomycin (VAN), patient-based data are limited (<50 patients) and appear to differ from data for healthy volunteers; as a result, it is not clear which free drug values should be used in pharmacodynamic analysis. The purpose of this study was to determine the PB of VAN and its variance in patient population with *S. aureus* infection.

**Methods:** Over a 3 m period (2008), pre dose VAN samples from 81 patients with confirmed staphylococcal sepsis were collected. PB was evaluated by ultrafiltration and concentrations of VAN were determined by fluorescence polarisation assay. Multivariate analysis was undertaken to investigate associations between the degree of PB and individual patient factors.

**Results:** The mean patient age was 66 y (range 24–98) and the mean total VAN concentration 9.8 mg/L (range 3.6–18.4 mg/L). The mean PB was 31.5% (95% CI 29.9–33.0%) with a range from 12.6–55.7%.

In multivariate linear regression, no relationship was found between age (P = 0.857), total VAN concentration (P = 0.866), serum albumin (P = 0.081) but a weak association (0.046% increase in PB per mL/min increase in eGFR) was seen between PB and eGFR (P = 0.016).

**Conclusion:** In patients with *S. aureus* sepsis PB is more consistent with healthy volunteer data (PB ≈ 30%) than to the limited patient data (PB ≈ 50%). Unlike earlier studies, we did not find a relationship between albumin and PB, perhaps related to differences in patient populations studied and/or severity of sepsis. We conclude that in a general hospital population, VAN PB may be lower than often believed. In pharmacodynamic calculations, PB values derived from infected patients relevant to the population being studied should be employed.

**Hepatitis from A – E**

**[P1990]** Clinical features of acute hepatitis A outbreak in Korea


**Objectives:** From April 2008 to August 2008, many cases of hepatitis A were notified in Korea. The aim of the present study was to investigate the clinical features of recent hepatitis A outbreak.

**Methods:** We retrospectively reviewed the medical records of 245 patients with acute hepatitis A from January 2006 to November 2008. Patients who 1) were positive to the other markers of acute hepatitis, 2) had underlying liver disease, 3) had a history of recent exposure of hepatotoxic drugs, and 4) had biliary obstruction on imaging studies were excluded. A total of 221 cases of hepatitis A were analyzed in this study. We compared clinical manifestations of hepatitis A between this outbreak (April 2008 to August 2008, Period 1) and before the outbreak (January 2006 to March 2008 and September 2008 to November 2008, Period 2).

**Results:** 113 cases of hepatitis A were detected during Period 1 (160.0 cases per 10,000 hospital admissions). The mean age was 32 and the proportion of adult patients older than 30 yr was increased from 62.7% during period 1, to 46.5% during period 2 (p = 0.024). The most frequently reported symptoms include fever, myalgia, nausea, abdominal pain and dark urine. The proportions of severe hepatitis A were 11.8% during period 1 and 6.1% during period 2, respectively. Eleven patients experienced acute renal failure and all of them completed recovered from renal failure (5 patients during period 1 and 6 patients during period 2). The laboratory tests (WBC count, Hemoglobin, platelet count, AST, ALT, total bilirubin, albumin and prothrombin time) at admission day were not significantly different. One patient developed fulminant hepatitis with hepatic encephalopathy and expired due to liver failure (Period 2). Others completely recovered without sequelae.

**Conclusion:** The majority of hepatitis A cases were completely recovered without sequelae although severe cases were slightly increased during outbreak period. The proportion of adult patients older than 30 yr has increased recently and this result is concordant with previously reported data.

**[P1991]** Cholestasis syndrome in viral hepatitis A

A. Petroc* (Plodio, BG)

**Objectives:** Cholestasis forms in viral hepatitis A (VHA) occurs rarely and is more correct to note the presence of cholestasis component in the jaundice cyclical form of the disease. Despite the marked patomorphological changes showing reliable infrahepatic cholestasis in VHA, clinical manifested cases are single and found mostly in adult patients.

**Material and Methods:** The study includes 820 patients with VHA, of which 400 cases of sporadic icteric form of the disease for five years period, 2002–2006 included as well as 220 children and 200 adult patients from outbreak in gypsy residential district of Plovdiv – 2006 y. Analyzed are eight parameters characterising cholestasis syndrome: max
Contemporary epidemiological characteristics of viral hepatitis A in Plovdiv region, Bulgaria

N. Yatev*, M. Staycheva, I. Batashki, M. Troyancheva, E. Kancheva (Plovdiv, BG)

Objectives: The aim of the work is: To examine whether during the preceding several years there have been any alterations in the epidemiology of VHA and whether there are substantial differences in the epidemiology of the disease regarding different groups of the population, living under different sanitary–hygienic conditions.

Methods: All clinical cases of VHA in 2005–2007 have been examined. The comprehensive method for epidemiological research and analysis has been used. A random group of 180 people has been examined to evaluate the seroprevalence of anti-HAV. The persons belong to 2 groups – living under bad and good sanitary–hygienic conditions. The serological research has been done with the ELISA method.

Results: The patients with VHA were 3 881. The morbidity rate varied from 52.74% to 69.4%. 68.24% of the patients are from Roman origin, living under bad sanitary–hygienic conditions and with low personal hygiene. In this group the highest morbidity was established in the childhood – above 60% in the age between 2 and 14 years. 31.76% are persons living under good sanitary–hygienic conditions. The majority of them (above 65%) are young people (20–30 years old), or older. The results from the seroepidemiological research (such research not having been performed in the country for more than 20 years) for the seroprevalence of anti-HAV are as follows: in the group of people living under bad sanitary–hygienic conditions, the examined persons are positive of anti-HAV in 90.32%. The persons living under good sanitary–hygienic conditions are positive in 44.83%.

Conclusion: Improvement in the sanitary–hygienic does not comprise all groups of the population in a particular region or country. Therefore it is necessary to conduct differentiated monitoring for control and prevention of the disease for the separate groups. We identify considerable differences in the epidemiology of VHA among the persons living under different sanitary–hygienic conditions. Reduction of the morbidity from VHA among people living under good sanitary–hygienic conditions results in increase in the risk of becoming infected as adults. Therefore, for them it is appropriately to conduct immunoprophylaxis if they are in contact with infected from VHA, or if they are traveling to a country endemic of the disease. This is a new recommendation, additional to the measures for prevention of the disease, which have thus far been supplied for the population of the country as a whole.

P1993 Seroprevalence of and occupational risk for hepatitis A among healthcare workers in Korean hospitals


Objectives: The cases of hepatitis A have been increasingly reported in Korea due to socioeconomic growth and a relatively low immunisation rate for hepatitis A in the general population. An outbreak of hepatitis A in Korean healthcare workers (HCWs) was recently reported. This study was performed to evaluate the serological epidemiology of hepatitis A among HCWs in Korean hospitals.

Methods: A total of 3,696 HCWs in 4 hospitals were tested for IgG antibodies against hepatitis A virus (HAV) using commercially available kits in 2008. Data including demographic characteristics, occupations, and departments were collected. Seroprevalence and risk factors were evaluated.

Results: Among 3,696 participants, 2,742 (74%) were women and the majority (96%) were in their twenties or thirties. The median age was 28 years (range, 19–68). Eighteen percent were medical doctors, 46% nurses, 10% nursing assistants, 11% technicians, and 15% workers in administration. Seropositivity for HAV significantly increased with age (p < 0.01); 2% for the age group <25 years, 15% for 25–29, 42% for 30–34, 76% for 35–39, and >90% for >40. Among the participants under the age of 40, anti-HAV seroprevalence was significantly lower in HCWs working in the metropolitan area and in the group of medical doctors. Statistically significant difference was not seen according to the departments.

Conclusion: Younger age, living in the metropolitan area, and working as a medical doctor were associated with lower anti-HAV seroprevalence in Korean HCWs. Immunisation for this group should be considered.

P1994 Modified epidemiology of hepatitis A in Italy. Prophylactic measures targeted on potential sexual transmission

R. Manfredi*, L. Calza (Bologna, IT)

Introduction: Outbreaks of hepatitis A have been recently observed worldwide, with special attention focused on homosexual men, i.e. drug users, and also heterosexual partners.

Methods: An observational survey of all hospitalisations due to hepatitis A occurred in the Bologna metropolitan area, performed from 1999 to mid-2007, was carried out.

Results: One hundred and 76 consecutive patients (p) with acute HAV hepatitis were hospitalised. From October 2002 to September 2004, hepatitis A largely prevailed over acute HBV-HCV-HEV hepatitis. Adult female p and children represented only 15.9% of overall cases. Among the 176 p with ascertained acute hepatitis A, the prevalence of immigrants increased over time, from 1–3 cases/year from year 1999 to year 2001, up to 14 cases in the 21 months elapsed from 2003 to September 2004 (p < 0.02). Even 121 out of 176 p (68.7%) were represented by male adults, aged 22–56y, who recognised unprotected homo-bisexuals contacts in the two months preceding hepatitis A onset in 83.5% of cases. Nobody was aware and/or underwent prior anti-HAV vaccination. Among the 121 adult males with acute hepatitis A, concurrent infections were found in 44 p (p <0.003 versus other p with HAV disease): chronic hepatitis B in 8 cases, hepatitis C in 19, syphilis in 11, and HIV in 11 p. The temporal trend of male adults admitted for hepatitis A showed a significant increase from 1999 to the first 9 months of 2004: a ~300% increase versus the year 1999, leading to a crude rate of 7.7/100,000 residents/year.

Conclusions: Despite the availability of anti-HAV vaccination, and information campaigns against the spread of STD and HIV, the epidemic of HAV recognizes an increased prevalence of homo-bisexual transmission. Epidemiological monitoring, targeted educational campaigns, and public health measures (such as an recommendation of immunoprophylaxis), may help contain the outbreak of hepatitis A by sexual route, and reduce the spread of other concurrent STDs.
Medical nursing students' knowledge and attitudes toward viral hepatitis in Turkey: a multi-centre cross-sectional study

Objectives: Medical nurses can potentially be exposed to the hepatitis B (HBV) or C virus (HCV) during their daily nursing tasks. Transmission can typically occur following a needle stick injury or mucosal exposure. It is necessary to inform them about viral hepatitis and to complete a full course of HBV immunisation regarding their prevention. The objectives of this study were to identify medical nursing students’ levels of knowledge on viral hepatitis and to ascertain their immunisation status and attitudes of hepatitis A virus (HAV) and HBV infection in Turkey.

Methods: This cross-sectional study was conducted on third and fourth-year nursing students located on all geographical regions of Turkey. A questionnaire comprising of 47 questions was applied (including 12 questions on sociodemographic factors, 22 about level of knowledge on HAV, HBV, HCV, 5 about immunisation status, 4 about risky behaviour history and attitudes in these situations). The knowledge score of participants was calculated by assuming that every correct answer=1 point, with maximum possible score 33.

Results: 1491 third and fourth-year nursing students were enrolled in the study. The mean age of the students was 21.4±4.6. The mean knowledge score of fourth-year students was significantly higher than third-year students (p<0.000). Students graduated from medical high school and who have a family member with chronic HBV infection had higher knowledge score than others (p<0.000 and p<0.01). 58.6% of the students rated their own knowledge level of viral hepatitis as intermediate and 29.9% of them rated as good. Knowledge scores of students were university education 93%, web pages 49.1%, high school education 41.4%, printed media 35% and TV/radio 27.2%. 85.3% of the participants had received HBV vaccine and 9.1% had received HAV vaccine. The percentage of students who signify themselves at increased risk of acquiring viral hepatitis was 97.3%. 28.1% of the students had needle stick injury and 5.4% had conjunctival exposure to blood. 98.2% of participants stated that they would take different precautions before performing routine tasks to patients with known infection.

Conclusion: Medical nursing students are at risk of acquiring blood-borne infections during their daily practice. This should be considered before management of education programmes about transmission of blood-borne infections and protection strategies.

Chronic hepatitis E in human immunodeficiency virus-infected patients living in south-eastern France

Objectives: Autochthonous hepatitis E is an emerging disease in industrialised countries. Chronic hepatitis E and even hepatitis E-associated cirrhosis have been recently described in organ-transplant recipients. This deserves increased awareness for HEV infection in immunosuppressed individuals. Limited information is available about HEV infection in HIV-infected patients (pts). We aimed at assessing the prevalence of anti-HEV antibodies (Ab) and HEV RNA in HIV-infected pts in Marseilles, south-eastern France.

Methods: Serum samples from 190 HIV-infected pts were tested for IgG/IgM anti-HEV Ab, and HEV RNA using in house real-time PCR and sequencing assays. HIV-1-infected pts were divided into 4 groups: 73 pts had a CD4-cells count (CD4cc) <50/mm² (mean, 27/mm²), 69 were recently diagnosed (in 2006) for their HIV infection (mean CD4cc, 386/mm²), 31 had liver cirrhosis (mean CD4cc, 375/mm²), and 17 were infected with HIV-2 (mean CD4cc, 481/mm²).

Results: IgG anti-HEV Ab were detected from 15 pts (8%); 5 of them were concurrently IgM anti-HEV Ab-. In addition, 2 other pts only harboured IgM anti-HEV Ab. IgG anti-HEV prevalence was 8%, 3%, 14%, and 13% in groups 1, 2, 3, and 4, respectively, without significant differences between groups. HEV RNA was detected from one HIV-1-infected pt. Retrospective analysis of sequential serum samples showed HEV RNA and IgM anti-HEV Ab detection from June 2006 until March 2007 (10 months), indicating chronic HEV infection. Further follow-up was not possible since the pt died due to cardiovascular disease. IgM anti-HEV Ab could be detected from June 2006 until March 2007, whereas testing for IgG anti-HEV was negative on all serum samples. The pt was a 44-year-old male who acquired HIV through intravenous drug use and was diagnosed in 1986 in the setting of Hodgkin lymphoma. In June 2006, the CD4cc was 157/mm², virological markers indicated past HBV and HCV infections, and ALT level peaked at 219 IU/L. The pt underwent several courses of chemotherapy for non-Hodgkin lymphoma from the end of 2006. The CD4cc progressively decreased to reach 27/mm² in March 2007. ALT were within normal range from September 2006. The patient did not recently travel abroad. HEV genotype was 3f.

Conclusion: HEV should be considered as an aetologic agent of hepatitis in HIV-infected individuals and might cause chronic infection in this population. HEV RNA and IgM anti-HEV Ab tests should be performed for reliable diagnosis of HEV infection.

Acute liver failure due to HEV in a long-term oral contraceptive treated patient
M. Rodríguez-Dominguez°, J. Graus, S. Fernández-Barredo, M.T. Pérez-García, F. García de la Hoz, M.L. Mateos (Madrid, Valencia, ES)

Introduction: HEV causes epidemics in developing countries but in industrialised there are autochthonous cases with different clinical and epidemiological features. In pregnant women the mortality rate is very high (up to 20%) mainly in the third quarter of pregnancy. Recently we have had a case of sudden hepatitis E in a patient treated with oral contraceptives. The sudden course may have been caused by a hormonal situation similar to pregnancy.

Case report: A 37 years old woman, was attended in our Hospital with an important increase in transaminases (AST 4522 U/L and ALT 3751 U/L), jaundice and coagulation changes (INR 1.5 rising to 3.7 at 48 hours despite of K vitamin treatment). Serological markers against HAV, HBV, HCV, HIV, EBV CMV and HHV were negative. IgG and IgM against HEV were positive by immunoenzymatic assay and confirmed by immunoblot, also RNA-HEV from two samples was detected by RT-nested-PCR. Genotype was 3f. A partial sequence of the strain isolated was obtained and compared to other human and swine strains. Phylogenetic analysis of a 260 bp long fragment belonging to the ORF2 revealed that this isolate showed a high homology (91.9-97.3%) with some Spanish human strains followed by other Spanish swine strains (86.9-94.2%). Compared to other human European strains, the closest homology was with some British strains (83.4-91.9%), and compared to other European swine strains, the highest homology was for Dutch strains (81.9-93%) followed by British (85.7-86.5%). Most of the mutations were found to be silent and did not result in significant differences at the amino acid level. The only previous data about toxins or drugs was oral contraceptives during last 20 years. Patient got better after admission taking routine antiepileptic treatment, K vitamin, acetil-cistein and antimicrobial prophylaxis. Liver transplant was not necessary because of the improvement of the patient.

Discussion: We think this is the first case of acute liver failure in a non pregnant women taking oral contraceptive, caused by HEV. An exhaustive interview was made in our patient in order to investigate risk factors that could justify the sudden course of hepatitis E. Only contraceptive oral treatment during 20 years could be found as risk factor. It has been suggested that estrogens and progestagens can simulate a pregnancy situation. Contraceptive treatment may be considered a risk factor for sudden course of hepatitis E.
Prevalence of HBV, HCV and HIV infections among obstetrics/gynecology patients


Introduction: Hepatitis B and C and the acquired immunodeficiency syndrome are major problems of public health worldwide. Infection of woman of childbearing age by these viruses is especially important due to the risk of vertical transmission in the perinatal period. The purpose of this study was to analyze results of routine serology screening for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) among patients of the Department of Obstetrics/Gynecology of our hospital.

Materials and Methods: The study included all patients admitted to the Department of Obstetrics/Gynecology during a 17 months period (from 7/2007 until 11/2008) and tested for markers of HBV, HCV and HIV infection. All data were retrospectively retrieved from laboratory records. Testing for HBsAg, anti-HBc, IgM anti-HBc, anti-HBs, HBeAg, anti-HBe, anti-HCV and anti-HIV was performed by using the NCBI online Genotyping tool (BioRad) Western Blot assays were used for the confirmation of HCV and HIV positivity.

Results: Among 1,403 women that were examined for HBV infection, 197 (14%) had been exposed to the virus (anti-HBc positive). Past infection (HBsAg negative) was detected in 171 (12.2%), while 26 (1.8%) suffered from chronic infection (HBsAg positive). No woman exhibited signs of acute disease (IgM anti-HBc negative), whereas 282 (20%) were either vaccinated or had a past infection with undetectable HBeAg titers (anti-HBe negative/anti-HBs positive).

Chronic infected patients were all HBsAg negative and anti-HCV negative; at least 38% of them were immigrants from other countries. Among 1,648 women examined for anti-HCV, only 9 (0.5%) were confirmed as positive. Finally, all 1,549 women that were tested for anti-HIV were negative.

Conclusions: The prevalence of HCV infection is low between women hospitalised in the Department of Obstetrics/Gynecology of our hospital. However, the higher prevalence of chronic HBV infection and the low frequency of anti-HBs positivity imply that intensification of vaccination programs is required. Finally, the predominance of the ‘vaccine escape’ mutation G145R.

Molecular epidemiology of hepatitis B virus infection among chronic carriers in Greece, 2000–2007

A. Fylaktou, D. Papaventzis, L. Skoura*, M. Dououdaki, N. Malisiovas (Thessaloniki, GR)

Objectives: Hepatitis B virus (HBV) infection is a global health problem affecting >13% of the world’s population. Greece is considered an intermediate endemic area, where genotypes A and D predominate. The purpose of this study was to investigate HBV genotypes and HBsAg subtypes among HBV chronically infected patients, as no previous genotype data were available from Greece.

Methods: Serum samples from 136 HBsAg (+) patients were tested (Jan 2000–Dec 2007). Viologic and biochemical markers (serum ALT) used in HBV monitoring were analyzed. HBsAg, anti-HBs, HBeAg and anti-HBe were measured by commercial immunoassay (AxSYM or ARCHITECT, Abbott Diagnostics, France). Serum HBV DNA was analyzed quantitatively with Cobas Amplicor (Roche Diagnostics, Basel, Switzerland). HBV genotypes were studied by partial sequencing of the S gene, containing the HBsAg “a” antigenic determinant. Genotyping was performed by using the NCBI online Genotyping tool and phylogenetic analysis. Nucleotide sequences were aligned pair wise with ClustalW and phylogenetic trees were constructed by the neighbour-joining method. Statistical significance was estimated by bootstrap analysis. The sequences were also used to predict the HBV HBsAg subtype.

Results: In 6/136 (4%) patients, coexisting HBsAg and antiHBs were found. Forty-six of 136 (34%) were HBsAg (+), 86/136 (63%) were anti-HBe (+), and 4/136 (3%) were HBsAg (+)/anti-HBe (+). Mean ALT was 238 IU/L, and HBV-DNA levels ranged from 8.2 × 10^5 – 11.5 × 10^7 copies/ml. Agreement between the two genotyping methods was found in all cases and a HBV genotype was assigned to all samples. Genotype D was almost exclusively prevalent (133/136=98%). Viral groups D/ayw2 (73%) and D/ayw3 (25%) were predominant. Group A/adw accounted for the 1% of cases. Strains from genotypes B and C were exclusively found among Chinese immigrants (1%). Single or multiple point mutations were found in 35 cases (26%). Some of the most common mutations occurred in amino acid positions 129, 133, 134, 144, and 145, including the ‘vaccine escape’ mutation G145R.

Conclusion: This was the first study presenting data regarding HBV genotypes in chronic HBV carriers in Greece. Our data confirmed that genotype D predominates in the Mediterranean basin. HBsAg escape mutants were found highly prevalent. Epidemiological monitoring of occult HBV is essential for HBV vaccine designing and for diagnostic, transplantation, blood banking, and haematological health services.

Prevalence of sexually transmitted diseases among female patients presenting with hepatitis B virus and human immunodeficiency virus infections in a Nigerian teaching hospital

O. Onigbogi*, O. Ojo (Ibadan, Lagos, NG)

Objectives: The prevalence of HIV infection has been on the increase in Nigeria in recent times. HIV-positive patients in our setting sometimes have hepatitis B virus (HBV) infection co-existing in them. Female patients are sometimes at particular risk because of the co-existence of other sexually transmitted infections (STIs) with these two viral infections. The aim of the study was to determine prevalence of sexually transmitted infections among female patients attending the clinic in a Nigerian teaching hospital. The clinic also serves as the reference centre for STIs in our region of the country.

Methods: A retrospective review of 221 case notes of patients attending the clinic from January 2001 to December 2007 were analyzed by utilising an on-going observational database at the STI clinic. Records of sexually transmitted diseases were based on clinical assessment and laboratory diagnosis as recorded in the case notes. Rate ratios, comparing prevalence rates (number of infections among the women surveyed) were calculated.

Results: The age of the women surveyed ranged from 18 to 54 years. Twenty-two (10%) of the patients were found to have clinical and laboratory evidence of several other STIs co-existing. Fifty patients (38%) had clinical and laboratory evidence of single-occurring STIs. Sixteen respondents (31%) had gonorrhoea infection, 14 (29%) had chlamydiasis, 13 (25%) had candida infection while 7 respondents (13%) had trichomoniasis.

The survival rate among women with STIs with HIV and HBV co-infection rose from 2.28 to 3.12 in the last 2 years of the review (Rate ratio = 3.15; 95% confidence interval (CI) = 1.31–7.44; p = 0.0002). The survival rate among women with STIs with HIV and HBV co-infection rose from 2.28 to 3.12 in the last 2 years of the review (Rate ratio = 3.15; 95% confidence interval (CI) = 1.31–7.44; p = 0.0002).

Conclusion: There was been a significant rise in the incidence of co-existing multiple STIs in the years 2005 and 2006. This coincides with the introduction anti-retroviral drugs in the hospital with greater survival rates and increased attendance and testing. Care providers should therefore be more vigilant for co-existing STIs and more aggressive in their management especially among HIV-positive women because of the peculiar nature of such infections when they also co-exist with HBV.

Occult hepatitis B infection in Tunisian pregnant women

H. Hannachi, O. Bahi, S. Mhalla, H. Triki, J. Boukaida (Sousse, TN)

Objective: In Tunisia, the national screening programme for identification of hepatitis B virus (HBV) infection in pregnant women is
based on HBs antigen (HBsAg) test. HBV-DNA in the serum can be detected in the absence of HBsAg in case of occult hepatitis B infection. The objective of this study is to evaluate the prevalence rate of occult hepatitis B in healthy pregnant Tunisian women.

Methods: During one year period (September 2007 to September 2008), a total of 2709 pregnant women were prospectively screened for HBsAg and anti-HBc antibody (anti-HBc). Negative HBsAg sera were systematically tested for anti-HBs antibody (anti-HBs). Detection of HBV-DNA was performed for ‘anti-HBc isolated’ sera. The presence of HBV genome was tested by a single-step PCR in Pre-S gene and a nested PCR in X region. The sensitivity of the PCR assays was evaluated using samples with known viral load.

Results: Seroprevalence of positive HBsAg was 4%. Vaccinal immunity was detected in 3% of HBsAg negative women. Anti-HBc was detected in 22% of pregnant women (including HBsAg positive women). Anti-HBc was associated with anti-HBs in 13.5% and isolated in 4.5% of total pregnant women. Among HBsAg isolated anti-HBc, isolated anti-HBs concentration varied between 5 and 10µM/mL in 19 women. Detection of HBV-DNA was performed in 98 cases (with anti-HBs lower than 5µM/mL) and was positive in three cases (3%) by the two PCR assays. Sensitivity of HBV-DNA detection was 10^{3} copies/mL.

Conclusion: occult hepatitis B can be misdiagnosed by selective HBsAg screening in pregnancy. This can lead to a lack of appropriate prophylaxis in newborns. Anti-HBc antibody should be tested routinely on pregnant women especially in a country of intermediate endemicity for HBV infection. More effort is needed for HBV vaccination strategies in Tunisian women.

P2002 Prevalence of hepatitis B genotypes and resistance mutations in patients with chronic hepatitis B infection

A. Gutierrez,*, I. Viciana, L. Mora, A. Infante, E. Clavijo, V. Garcia, A. Pinedo (Malaga, ES)

Introduction: Nearly 400 million people worldwide suffer from chronic hepatitis B virus (CHB) infection. HBV strains can be classified into eight genotypes, designated A-H with distinct geographical distribution. Several drugs have been approved for the treatment of CHB and others already used as antiretroviral agents show anti HBV activity and most likely will soon be approved as therapy for hepatitis B. However, the emergence of HBV resistance mutations can reduce or annul the activity of the drugs.

Objective: The aim of this study was to estimate the distribution of genotypes of HBV and the prevalence of drug resistance mutations in patients with chronic hepatitis B attending in several centres across Malaga (Andalucia).

Methods: Genotype and drug resistance mutations were determined by population sequencing using TruGene HBV genotyping kit (Siemens Healthcare). For nucleic acid isolation we used the automatic system MagnaPure (Roche) in patients with detectable viral load of HBV.

Results: A total of 143 patients with chronic hepatitis B infection were recruited at 3 care centres during years 2005-2008. Of these, 70.6% were male and 29.4% female with the following characteristics: median age 46 years, median viral load 1.710^{5} copies/mL and unknown factors for HBV infection in 66.1% following for vertical transmission in 25.2%. Genotype distribution was: Genotype A: 36 (25.4%): A2 90%, A1 10%, Genotype D: 86 (60.6%), Genotype E: 5 (3.5%), Genotype F: 3 (2.1%). Genotype G: 1 (0.7%). Confection with several genotypes were not detected. 80 patients (56%) were under antiviral treatment and in 32(40%) resistance mutations were selected. The changes in the HBV polymerase were L180M and M204 I/V in 30 cases(21.1%) with cross resistance to Lamivudine, Emtricitabine and Telbivudine and N236T and A181V/T which confer resistance to Adefovir in 5(3.5%) of cases. Mutations associated with Entecavir or Tenofovir were not selected and transmission of drug resistant strains were not detected.

Conclusions: The genotype predominant in our area is genotype D following for genotype A. The rate of emergence of HBV resistance mutations is significant in patients on going treatment, mainly those associated with Lamivudine. The availability of antiretrovirals with potent antiHBV activity, in particular Entecavir and Tenofovir, appears to modify this poor outcome in recent years.

P2003 Delta hepatitis infection in patients with chronic hepatitis B infection in Isfahan, Iran


Introduction: Hepatitis D virus is a defective RNA virus dependent on hepatitis B virus infection for its replication and expression. Infection with HDV can occur simultaneously with acute HBV infection or may be superimposed on chronic HBV infection. It is known that coexistent infection with HBV infection with HDV tend to accelerate the progress of chronic HBV infection to chronic hepatitis, cirrhosis and hepatocellular carcinoma. This study was carried out to determine the seroprevalence of HDV among chronic hepatitis B patient in Isfahan in year 2008.

Materials and Methods: This study was done at first 7 month of year 2008 and was cross-sectional and done among 347 chronic hepatitis B patients that got along more than six months from their diagnosis and had medical file at infectious disease and tropical medical research centre. All Case were evaluated for the presence of total HDV Ab using Elisa (Diaopro-Italy). Chi-square and Fisher test and T-student was used to determine the relationship between independent variables and HDV seropositivity.

Results: Of 347 cases 246 were male (70.9%) and 101 were female (29.1%). 232 were Anti HBe positive (66.9%) with mean age 41.2±11.8 and 88 were HBe Ag positive (25.4%) with mean age 34.6±12.8. Anti-HDV Anti body was positive in 6 male (2.4%) and in 4 female (4%) (P=0.48). Anti HDV Ab was positive in 8 Anti HBe positive (3.4%) and in 2 HBe Ag positive (P>0.5). The mean age in HDV Ab positive patients was 43.9±12.4 years. Mean long duration disease in HDV Ab positive patients was 3.5±2.7 years. The total seroprevalence of HDV Ab in hole patients was 2.9%.

Conclusion: This Findings show that HDV infection is endemic in Isfahan province and its prevalence is high in HBe Ab positive patients than HBe Ag positive patients. We found no relationship between HDV seropositivity and age, sex, long duration of disease and kind of diagnosis and transmission. The seroprevalence of hepatitis D in this study at compare previous study in Iran is nearly equal.

P2004 Prevalence of blood-borne viruses amongst antenatal clinic patients and blood donors in a tertiary referral hospital in Oman

H. Schuster,*, E. Sererbour, J. Nograles, I. Al-Belushi, K. Hassan (Muscat, OM)

Objective: Recent data published for the Gulf region on chronic hepatitis B infection in pregnancy suggested a prevalence of 7.1% for Omani woman. The sample population in this report was small and we intended to re-evaluate the prevalence of Hepatitis B infection for pregnant woman attending antenatal care at Sultan Qaboos University Hospital. We compared the data for Hepatitis B and HIV infection and also assessed the Hepatitis C infection rate amongst of a blood donor population at the same institution.

Methods: The laboratory information for HIV, HBV and HCV infection data was obtained from the laboratory information system for the period from 01/07/2006 to 31/12/2008. The data was analysed with MExcel and Minitab.

Results: Amongst 3142 pregnant woman a prevalence rate of 7.07% was found for chronic HBV infection. 0.12% of chronic infections in pregnancy were of high infectivity. For 0.34% neither Hepatitis B e antigen nor anti-Hepatitis B e antigen antibody could be detected. There was a significant difference between the HBV infection rate amongst blood donors (n = 6726) and pregnant women 2.81% vs. 7.07 respectively. The HIV rate was found to be 0.17% for pregnant women and amongst blood donors 0.01%. Only blood donors were tested for HCV infection and a rate of 0.45% was seen.
Amongst blood donors anti-Hepatitis core antibody was detected in 18.8% of 745 donors without Hepatitis B surface antigenaemia. Hepatitis core antibody testing had only been introduced into blood bank screening in November 2008.

**Conclusions:** The rate of hepatitis B infection in Omani pregnant women remains at an intermediate endemicity level. The lower blood donor prevalence may be explained by a pool of stable donors although ad hoc blood donations are relatively common. The HIV rate amongst pregnant women is very low and even lower in the blood donor population. The HCV infection rate amongst blood donors is very low in the Sultanate.

A high rate of anti-Hepatitis B core antibody presence was found for blood donors without Hepatitis B surface antigenaemia. Because of the danger of occult HBV infection in such cases strategies for effective testing have to be found in order to maintain sufficient quantities of blood products.

Prevalence of blood borne viruses amongst antenatal clinic patients and blood donors in a tertiary referral hospital in Oman from 01/07/2006 to 31/12/2008.

<table>
<thead>
<tr>
<th></th>
<th>Antenatal clinic</th>
<th>Blood donors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total patients</td>
<td>3142</td>
<td>6762</td>
</tr>
<tr>
<td>Total tests</td>
<td>7002</td>
<td>10100</td>
</tr>
<tr>
<td>Age range at first visit (yrs)</td>
<td>12–55</td>
<td>17–65</td>
</tr>
<tr>
<td>Mean age at first visit (yrs)</td>
<td>28.8</td>
<td>28.1</td>
</tr>
<tr>
<td>HBsAg positive patients</td>
<td>222</td>
<td>190</td>
</tr>
<tr>
<td>Prevalence rate (%)</td>
<td>7.07</td>
<td>2.81</td>
</tr>
<tr>
<td>Hepatitis B carriers, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>high infectivity</td>
<td>4 (0.12)</td>
<td>14 (0.21)</td>
</tr>
<tr>
<td>low infectivity</td>
<td>208 (6.61)</td>
<td>169 (2.5)</td>
</tr>
<tr>
<td>HBsAg and anti-BeAg antibody negative, n (%)</td>
<td>10 (0.34)</td>
<td>7 (0.1)</td>
</tr>
</tbody>
</table>

| **HIV**           |                  |              |
| Total patients    | 3015             | 6690         |
| Total tests       | 7006             | 10272        |
| Age range at first visit (yrs) | 13–53           | 17–65        |
| Mean age at first visit (yrs) | 28.9             | 28           |
| HIV positive (confirmed by Western Blot), n | 5 1             |              |
| Prevalence rate (%) | 0.17             | 0.01         |

| **HCV**           |                  |              |
| Total patients    | 6725             |              |
| Total tests       | 10272            |              |
| Age range at first visit (yrs) | 17–65           |              |
| Mean age at first visit (yrs) | 28             |              |
| HCV positive (confirmed by HCV RIBA), n | 30 |              |
| Prevalence rate (%) | 0.45             |              |

**Results:** From 44 cases, 7 sera (2.1%) remained negative for HBcAb using B kit and 9 sera (2.8%) respectively with C test. For 2 discordant results the samples absorbances from B producer were around to cut-off values and we can consider the results as “low positive” and “echivocal” respectively. The lack of HBsAg and/or HBcAb in 5 HBsAg positive patients suggested a false positive result for this marker; we certified this possibility by confirmatory test from the same producer, A respectively. Because of these discordant results and in the lack of another conformatory test we cannot calculate the sensitivity and specificity of the tests. A further determination by biological molecular techniques for these cases will be neccessary. The low performance of A producer kit for HBcAb indicates this kit as being inappropriate and it strongly recommends B and C producers kits for HBcAb. The test results obtained using A test cannot be explained by the existance of immunosuppression or by mutant variants of HBV in this high percent (13.7%), this can be a consequence mainly due to a sensitivity default of analytical assay.

**Conclusion:** The low sensitivity of A producer kit for HBcAb strongly recommends to test this marker using more sensitive kits such as those from B or C producer. The true negative HBcAb cases can be explained by mutants strains and/or by associated pathology that induces a low immunosuppression; a future testing using sequence analysis for pre-core/core region of DNA/HBV is recommended.

**Atypical serological pattern in chronic hepatitis B virus infection: a real problem or a sensitivity default of the analytical kit?**

L.S. Iancu*, C. Durnea, A. Nastase, E. Enache, C. Manciuc, G.I. Pandele (Iasi, RO)

Chronic carriage of hepatitis B virus (HBV) is sometimes associated with an atypical serological pattern characterised by the presence of hepatitis B surface antigen (HBsAg) without antibodies to the HB core antigen (HBcAb).

The objective of our work was to study the reasons of the lack of HBcAb in HBsAg positive chronic HBV infected patients.

**Methods:** In 2006 and 2007 we have detected 5 HBsAg positive patients suggested a false positive result for this marker; we certified this possibility by confirmatory test from the same producer, A respectively. Because of these discordant results and in the lack of another conformatory test we cannot calculate the sensitivity and specificity of the tests. A further determination by biological molecular techniques for these cases will be neccessary. The low performance of A producer kit for HBcAb indicates this kit as being inappropriate and it strongly recommends B and C producers kits for HBcAb. The test results obtained using A test cannot be explained by the existance of immunosuppression or by mutant variants of HBV in this high percent (13.7%), this can be a consequence mainly due to a sensitivity default of analytical assay.

**Conclusion:** The low sensitivity of A producer kit for HBcAb strongly recommends to test this marker using more sensitive kits such as those from B or C producer. The true negative HBcAb cases can be explained by mutants strains and/or by associated pathology that induces a low immunosuppression; a future testing using sequence analysis for pre-core/core region of DNA/HBV is recommended.

**Are isolated anti-Hbc blood donors in a high-risk group? The detection of HBV DNA in isolated anti-Hbc cases with NAT nucleic acid amplification test based on transcription-mediated amplification**


**Aim:** Hepatitis B virus (HBV) can be transmitted by blood transfusions even so using serological tests having high sensitivity and specificity. We aimed to detect HBV DNA in isolated anti-HBC donors and show if they have transfusion risk or not.

**Method:** We investigated Anti-Hbc and Anti-HBs in serum samples of 12858 HBsAg negative blood donors who were applied to the Turkish Redcrescent between June 2007-October 2008 by the Micro ELISA kit (Hepanostica ultra HBc, Bio Meriux, France), Anti-Hbc and Anti-Hbs positive cases were omitted. We used Procleix ulitio (Chiro, USA) test kit (Chiron Tigris automated instrument was used) based TMA (Transcription Mediated Amplification) for NAT study in Anti-Hbc positive and Anti-Hbs negative serum samples. The discrimination of HBV in NAT positive serum samples were performed by Procleix Discrimination (Chiro, USA) test.

**Results:** 2748 (21.4%) Anti-Hbc seropositivity were detected in 12852 HBsAg(−) serum samples. 23.5% Anti-Hbs negativity was detected in 2748 Anti-Hbc positive serum samples. On the other hand, 5.1% isolated Anti-Hbc positivity [HBsAg(−), Anti HBc(+), Anti-HBst(−)] were detected in 12852 HBsAg(−) serum samples. 0.091% and 0.047% HBV DNA positivity were detected in isolated Anti-Hbc positive serum samples and HBsAg(−) serum samples, respectively.

**Conclusion:** As a result, even we have detected one (1) HBV transmission in every 2142 blood transfusion by HBsAg screening tests; we suggest that it is not necessary to add additional tests to detect isolated Anti-Hbc for routine purposes in Blood Banking. The reasons are higher negativity rates (99%) of isolated Anti-Hbc serum samples and the rejection of blood donors with Anti-Hbc positivity and also additional tests (anti-Hbc) are not being cost-effective.

**Twenty-year follow-up of vaccination against hepatitis B in patients with chronic renal failure**

L. Roznowsky, L. Kabieszowa, L. Hoazkoua*, A. Kloudova, J. Mrzek, I. Lochman (Ostrava, CZ)

**Objectives:** The vaccination against hepatitis B can considerably decrease number of hepatitis B virus infection among patients with
chronic renal failure. Breakthrough infections and anti-HBs antibodies response after immunisation were investigated in patients from 4 dialysis units in the north-eastern part of the Czech Republic.

Methods: Active immunisation against hepatitis B was commenced in 1988. The number of immunised patients with renal failure gradually increased and the group included 1163 patients in January 2009. Of these patients, 522 died during investigation. The vaccination schedule was 0, 1, 6 moths for pre-dialysis patients and 0, 1, 2 months for dialysis patients and patients with renal transplantation. Plasma-derived or recombinant vaccines (since 1990) were administered intramuscularly. Each vaccine contained 40 microgram of HBsAg, but for pre-dialysis patients only 20 microgram till 1998. The immunisation schedules were completed in 806 patients.

Blood samples were obtained 6 weeks after third or next dose of vaccine and biannually thereafter. Samples were tested by ELISA methods for HBsAg, anti-HBs and anti-HBc. The patients without protective anti-HBs antibodies after vaccination were once or twice re-vaccinated. The patients with vanishing of anti-HBs antibodies were also re-vaccinated.

Results: The new HBsAg positive status was proved in 28 dialysis patients, most of them suffered from acute hepatitis B. Breakthrough infections were more frequent after initiation of vaccination programme, 27 of them were in period 1988–1994, the latest breakthrough infection was proved in 2000. Asymptomatic infections with new appearance of anti-HBc antibodies (two or more consecutive positive results) were detected only in 8 patients. The anamnestic response (double increase of anti-HBs without revaccination) was observed in 91 patients. Two or more anamnestic responses were recorded in 15 of them.

Anti-HBs antibodies after vaccination were investigated in 728 patients. Protective anti-HBs levels were proved in 150 of 728 patients (48%) after 3 doses of vaccine and in 451 (62%) or 504 (69%) patients after fourth or fifth dose of vaccine.

Conclusion: Long-term vaccination considerably reduced hepatitis B incidence in 1163 patients with chronic renal failure, but only 69% immunised patients developed protective anti-HBs level after 5 doses of vaccine.


P2009 Evaluation of anti-HBs titre in surgeons vaccinated for HBV I. Karimi*, R. Sherkat, M. Rostami (Isfahan, IR)

Objectives: Health care workers including surgeons are frequent subjects of exposure to blood and infected tissues of HBsAg positive patients. A complete course of vaccine usually gives protection in majority of time but it may cause a false sense of security which in case of vaccine failure infection is inevitable. So it is essential to measure the level of antibody after vaccination. We designed this study to estimate the rate of people who has protective response to vaccine.

Methods: This descriptive and prospective study designed to know the response rate in surgeons after vaccination against Hepatitis B. 99 Medical surgeons who had history of vaccination were chosen conveniently. They filled a questionnaire regarding demographic and individual data.5 ml of venous blood was taken and after separation of 2 ml of serum, anti HBsAg measured by ELISA method using Hepanostica Anti-HBs kits from the Organon series.

Results: According to the kits instruction titers <10 IU/l are negative and >10 IU/l positive. Strongly positive is >100 IU/l.

Group 1: included 36 people who had received vaccine during recent 3 years. In this group 17 people had complete course of vaccination that 7 had titers less than 10 IU/l and another 10 were in range of positive titers.

Group 2: included 32 people who had received vaccine during recent 3 to 5 years. 24 people had complete course of vaccination that among them 8 had titers less than 10 IU/l and 14 were in range of positive titers and 2 were negative.

Group 3: included 41 who had received vaccine more than 5 years ago. 32 people had complete course of vaccination that among them 24 had positive titers and 5 had titers less than 10 IU/l and 3 were negative. Among 9 who had incomplete course of vaccination 2 were positive, 1 strongly positive and 6 were negative.

Conclusion: We had no case of non responder in those who had complete course of vaccination during 3 years from study. We had more negative titers in people who had completed the course between 3 to 5 years or more. This may be due to type of vaccines used in that period of time. Because of higher risk of infection in health care workers and primary and secondary failure to make antibody against virus in 40% we recommend strict adhesion to standard precautions even in everybody including vaccinated people. We need to say that, if all the subjects had received the same generation of vaccine we were able to make better and more precise comments.

P2010 Decline in HBsAg level during treatment with PEGASYS® is significantly associated with post-treatment response in patients with HBeAg-negative disease P. Marcellin*, M. Brunetto, F. Bonino, S. Halaziyannis, H.P. Kapprell, D. Messinger, R. Bartra (Clichy, FR; Pisa, Milan, IT; Athens, GR; Wiesbaden, Mannheim, DE; Basel, CH)

Objectives: A 48-week course of treatment with PEGASYS® (pegylated interferon alfa-2a with dual immunomodulatory and antiviral properties), is able to induce a sustained virological response 6 months post-treatment in around one third of patients with HBeAg-negative disease. Such patients have an increasing chance of developing HBsAg
clearance during longer term follow-up – the closest outcome to cure in patients with this chronic condition. In a phase 3 study comparing PEGASYS® with lamivudine, PEGASYS®lamivudine but not lamivudine monotherapy resulted in significant decline in HBsAg levels by end of 48 weeks treatment in chronic hepatitis B patients. Thus, it was hypothesised that monitoring HBsAg levels during the course of treatment may be a useful marker to identify the patients most likely to benefit from this treatment approach.

Methods: This was a retrospective subanalysis of patients treated with PEGASYS®lamivudine in the phase 3 study. A total of 230 patients treated with PEGASYS®lamivudine entered a roll-over observational follow-up study to determine long-term response: HBV DNA suppression (<400 copies/mL) or HBsAg clearance 4 years post-treatment. HBsAg levels were measured (Abbott Architect HBsAg Assay) in all available sera pretreatment, and at weeks 12, 24, 48 and 72. We compared the HBsAg decline during therapy in patients with and without an HBV DNA response 6 months post-treatment to determine if HBsAg levels during therapy may be indicative of response.

Results: A high proportion of patients with an HBV DNA response <400 copies/mL 4 years post-treatment had cleared HBsAg at this time (66%, 25/38). Mean baseline HBsAg levels in patients treated with PEGASYS® were significantly lower in patients with a sustained virological response (HBV DNA <10,000 copies/mL) 6 months post-treatment than in those without (Table). HBsAg levels declined throughout treatment with PEGASYS®. By treatment week 24 HBsAg decline was significantly greater in patients with a sustained response vs those without (Table).

Table 1. Analysis of HBsAg decline in patients with and without virological response (HBV DNA <10,000 copies/mL 6 months post-treatment): PEGASYS® monotherapy

<table>
<thead>
<tr>
<th>HBsAg (log10 IU/mL), mean±SD (N)</th>
<th>Respondera</th>
<th>Non-responder</th>
<th>P-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4.20±0.70 (61)</td>
<td>4.45±0.48 (71)</td>
<td>0.0204</td>
</tr>
<tr>
<td>Decline from baseline to week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.19±0.52 (36)</td>
<td>0.08±0.43 (49)</td>
<td>0.2854</td>
</tr>
<tr>
<td>24</td>
<td>0.69±1.14 (52)</td>
<td>0.12±0.66 (60)</td>
<td>0.0052</td>
</tr>
<tr>
<td>48</td>
<td>1.01±1.27 (61)</td>
<td>0.28±0.64 (68)</td>
<td>0.0005</td>
</tr>
<tr>
<td>72</td>
<td>0.83±1.23 (58)</td>
<td>0.23±0.52 (67)</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

aResponse: HBV DNA <10,000 copies/mL 6 months post-treatment.

bWald chi-square test for association between response and change in HBsAg from baseline.

Conclusion: Patients who achieve a sustained virological response following PEGASYS® treatment have a high chance of clearing HBsAg during longer term follow-up. Monitoring HBsAg levels during PEGASYS® therapy can help identify the patients most likely to achieve a virological response 6 months post-treatment and clear HBsAg and could help optimise the management of PEGASYS® therapy in patients with HBsAg-negative chronic hepatitis B.

**P2011 Lamivudine treatment for acute severe hepatitis B**

A. Verhaz (Banja Luka, BA)

Objectives: Lamivudin has been approved for the treatment of chronic hepatitis B but experience with lamivudin treatment for acute severe hepatitis B is still limited. Fulminant hepatitis develops in 1% of patients with acute hepatitis B. Severe acute hepatitis B in immunocompetent patients may progress to fulminant hepatitis and death.

Aim: To evaluate the efficacy of lamivudine for the treatment of acute severe hepatitis B virus infection in immunocompetent adults in Clinic for infectious diseases Banja Luka.

Patients and Methods: In the period of 2006–2008 years, 9 immunocompetent patients (3 women, 6 men, age 30–77 years) with severe acute hepatitis B were treated with lamivudin. All 9 patients fulfil at least two of the criteria for severe acute hepatitis B infection: 1. hepatic encephalopathy; 2. total bilirubin 210 micromole per litre; and severe coagulopathy (international normalised ratio-INR was 4.5±6.4 or prothrombin time – PT <40%). All patients had evidence of severe hepatocytelysis. Nine patients had rapid increase of total bilirubin and contemporary decrease of alanine aminotransferase level, which escalate risk of development of fulminant hepatitis B. All patients received lamivudin at a dose 100 mg per day.

Results: Eight patients responded well to the treatment and their biochemical parameters improved rapidly. Within 1–6 months, the HBsAg was undetectable in 8 out of 9 investigated patients. Protective anti-HBs antibodies developed in 8 of them in 2–14 months. The corticosteroid therapy was short-term in 2 of 9 patients. One patient developed fulminant hepatitis B and died 4 days after the lamivudine therapy was initiated. Lamivudine treatment was well tolerated in all patients.

Conclusion: Lamivudin induces a prompt clinical, biochemical and serological response in immunocompetent patients with severe acute hepatitis B. Early treatment with lamivudine probably decreases the risk of progression to fulminant hepatitis in patients with severe acute hepatitis B.

**P2012 Patterns of resistance mutations in patients with chronic infection by hepatitis B virus treated with lamivudine and/or adefovir-dipivoxil**

B. Puche, J.C. Palomares*, M.C. Nogales, B. Figueruela, E. Suarez, E. Martin-Mazuelos (Seville, ES)

Objectives: Treating chronic hepatitis and liver cirrhosis caused by hepatitis B virus (HBV) infection with nucleos(t)ide analogues (NA) is very challenging due to the appearance of resistance mutations. Our aim was to study the prevalence of these mutations after viral breakthrough in patients treated with lamivudine (LMV) or adefovir-dipivoxil (ADV) in Occidental Andalusia.

Methods: 37 patients were studied, 89% were HBsAg negative, 70.3% were diagnosed of chronic hepatitis and 29.7% of liver cirrhosis. General follow-up was carried out measuring viral load (COBAS Ampliprep-Taqman, Roche Diagnostics) and serum ALT levels before treatment and every 3 months and genotype and resistance mutations were determined before treatment and in cases where viral breakthrough was observed. The latter were determined by direct sequencing of the surface and polymerase gene, respectively, using the Trugene HBV Genotyping kit (Siemens Medical Solutions).

Results: Genotype D was observed in 75.7% of our patients, 18.9% A, 2.7% B and 2.7% F. All were wild-type (WT) strains before treatment. Twenty-two patients were initially treated with LMV. After a mean of 2.5 years, 20 (91%) presented viral breakthrough. LMV was substituted for ADV in 15 cases, entecavir (ETV) in one and ADV was added in 4. Resistance mutations to LMV were developed in an 80% of the cases, L180M+M204V in 10 (one accompanied by V207I) and M204I in one. Five patients (33.4%) treated with ADV presented viral breakthrough after a mean of 2 years. ADV was substituted for tenofovir (TDF) or LMV-TDF in 2 cases each and ETV in one. Resistance mutations to ADV were developed in 3 cases, one A181V and 2 A181V+N236T. On the other hand, 15 patients were initially treated with ADV. After a mean of 2 years, 4 (26.7%) presented viral breakthrough. ADV was changed to TDF in 2 cases and LMV was added in 2. Resistance mutations to ADV were observed in 1 patient (A181V). All studied patients are currently treated with the last cited antivirals and no viral breakthrough has been observed in a mean of 10 months.

Conclusions: 1. Resistance mutation development is the major cause of viral breakthrough during treatment with NA, specially in LMV monotherapy and in non-naive patients treated with ADV. 2. The most frequent mutations involved in LMV treatment failure are L180M and M204V/I. 3. The most frequent mutations involved in ADV treatment failure are A181V and N236T.
**P2013** Failure to treat HBV with adefovir due to early selection of rtA181T mutation

M. Rodríguez-Domínguez*, J.M. Sánchez-Calvo, V. Moreira, G. Arranz, M.L. Mateos (Madrid, ES)

**Introduction:** The main goal of antiviral treatment against HBV is to abolish viral replication. Lamivudine is widely used but a high number of patients have no response to treatment due to the selection of HBV mutants, resistant to lamivudine during treatment. Therefore, adefovir-dipivoxil is the main alternative because it is less frequent to found resistant mutants of HBV. Recently we have had a patient with chronic HBV, HBeAg negative, in which treatment with adefovir get failed due to an early selection of a resistant mutant rtA181T.

**Case report:** A 47 years old female, asymptomatic, in a routine laboratory test was found to have a light increase in transaminases levels. Serological markers against hepatotropic viruses were studied founding a positive surface antigen for HBV (HBsAg) and a viral load (DNA-HBV) of $25 \times 10^6$ UI/mL, HBeAg was negative and anti-HBe was positive. No antibodies against HCV and HDV were found. Treatment with adefovir was started (10 mg/24h). During a one year period, viral load decreased but always remaining about $8−9 \times 10^4$ UI/mL. A year and a half after viral load increased to $7.4 \times 10^5$ UI/mL. Suspecting a failure due to resistant HBV, adefovir was changed to tenofovir (300 mg/24h). In a retrospective study of resistant mutations against lamivudine, adefovir, tenofovir and entecavir by a reverse-hybridation technique (INNO-Lipa DR v2 and v3, Innogenetics, Belgium) we found that the mutation rtA181T appears two months after the beginning of adefovir treatment. Also, we tested serum samples previous to adefovir treatment, and we did not found any other resistant mutation.

**Discussion:** rtA181T mutation is mainly associated with lamivudine resistant HBV from which susceptibility is reduced about three times. Against adefovir, this mutation can be considered secondary because it confers a low level of resistance in vitro. In our case, low levels of viral load were maintained during one year but never under $10^4$ UI/mL. The retrospective study of banked serum samples allowed us to confirm that the treatment failure was due to the early selection of rtA181T mutant of HBV.

**P2014** Molecular analysis of “a” determinant region of the S gene of HBV from healthy subjects with occult HBV infection in Istanbul


**Objective:** HBV DNA and AntiHBc antibody positivity without HbsAg is defined as occult HBV infection, which has been detected at varying rates according to the local prevalence of HBV infection. Although in subjects with occult HBV infection “a” determinant region containing a dominant B cell epitope for anti-HBs response shows higher amino acid substitutions compared to HbsAg positive carriers, the exact mechanism of occult HBV infection is not fully elucidated. In this study we aimed to determine the mutations in the “a” region of S gene in subjects with occult HBV infection.

**Methods:** Eighty three blood donors positive for anti-HBc antibody alone were screened for HBV DNA by PCR targeting S gene. Following the sequencing of the PCR amplicons, mutations with a potential of antigenic change in the “a” determinant region of the S gene were sought.

**Results:** Eight of 83 subjects (9.63%) were positive for HBV DNA. Based on the amino acid sequences between 101–180 all of them were ayw2 subtype and had no mutations in the “a” determinant region which might be related with antigenic change in the major B cell epitope.

**Conclusion:** Other mechanisms then the mutations in S gene of HBV may be involved in the occult HBV infections in our region with the exclusive predominance of the HBV genotype D.

**P2015** Genotype characterisation of hepatitis G virus isolates from Iranian patients infected with human immunodeficiency virus

A. Ramezani*, M. Mohraz, A. Aghakhani, M. Banfazl, A. Esalamifar, A.A. Velayati (Tehran, IR)

**Objectives:** Hepatitis G virus (HGV) infection is frequent in patients infected with the HIV due to similar transmission routes of these viruses. The aim of this study was to determine the rate of infection and genotypic characteristics of HGV in this population.

**Methods:** The presence of HGV RNA was determined in serum samples of 106 patients infected with HIV by reverse transcriptase-nested polymerase chain reaction. HGV genotypes were determined by direct sequencing. HbsAg, anti-HBs, anti-HCV, ALT, HIV viral load and CD4+ cells count were also tested in all subjects.

**Results:** The overall prevalence of HGV infection was 11.3% in HIV positive patients. There was no significant difference between patients with and without HGV infection regarding age, sex, route of transmission, viral load, ALT levels, HBV and HCV co-infection and...
Hepatitis from A – E

P2010 Accidental blood exposures among medical residents in Paris, France


Accidental blood exposure (ABE) exposes health care workers (HCW) including medical residents (MRs) to the risk of occupational infection. We aimed to determine the characteristics of ABEs in MRs in Paris, France.

An anonymous self-reporting questionnaire was administered electronically. A total of 350 MRs (33% from surgical disciplines) entered this survey. Median age was 27 years (range: 23–35), 32% were males. One hundred and eighty five MRs (52%) reported at least one ABE during their residency (median: 2, range 1–25), 53% of which occurred in operating rooms.

Sixty-nine percent of MRs followed the current procedures for local disinfection. ABEs were notified to the hospital administration by 62% of MRs, but only 51% of MRs referred to occupational medicine department. However, in 74% of cases, the serologic status of the index patient was investigated. Eight MRs received HAART, the most frequently prescribed combination being zidovudine, lamivudine and boosted lopinavir. None discontinued this treatment.

The most frequently reported concerns following ABEs were HIV infection (52%) and HCV infection (39%). HBV was not a major concern in this population with 54% of MRs being aware of their HBs antibody titres.

ABE is a major issue in HCW. Medical residents, although aware of the potential risks of blood-borne infections, behaved inappropriately in up to 33% of cases in this survey. Further educational programs should include MRs and not only senior HCW in order to improve individual behaviour when facing ABEs.

P2017 Healthcare personnel’s experiences with sharps/needle-stick injuries and preventive measures

M. Altiok, F. Kayurtar, S. Karacorlu, G. Ersoz*, S. Erdogan, H. Goke (Mersin, TR)

Objective: In today’s work environment health care personnel are at risk for infectious diseases from sharp instruments and needle sticks contaminated with patients’ body fluids.

This study was planned for the purpose of determining hospital and public health clinic health care personnel’s experiences with sharps/needle stick injuries and the preventive measures they take when injured.

Methods: The population of this descriptive and cross-sectional study was all 2532 health care personnel who worked at one university hospital, two state hospitals, and 54 public primary health care clinics in Mersin province. Using a random sampling method stratified by health care professions the goal was to reach all professional groups (357 physicians, 578 nurses/midwives, and 78 laboratory technicians). Data were collected by having the participants personally complete a data collection form. The forms were collected and Chi square test was used in the statistical analysis.

Results: A total of 956 (%37.7) of the health care personnel in hospitals and public health clinic health care personnel interviewed. In our study the injury rate was 479/1, %60.9 of those were injured by instruments contaminated by blood and the most (%89.2) had experienced needle sticks. The majority of the injuries occurred at the patient’s bedside; a significant percentage had been injured using incorrect practices of recapping a needle and removing a needle from a syringe and while disposing of sharps/needles in the sharps container. Injuries occurring while disposing in the sharps container happened the most often in public health clinics. In our study the most injuries occurred in nurses/midwives; the percentage of personnel having received the hepatitis B vaccination was similar in physicians and nurses/midwives and was low in laboratory technicians (P = 0.006, χ² = 10.378). Only %12.7 of the health care personnel had reported their injury.

Conclusion: In this research a high percentage of blood contaminated sharps/needle stick injuries was found, a low percentage of injuries were reported, and a high percentage of personnel had received the Hepatitis B vaccination. Although the level of vaccination was encouraging the injuries from incorrect practices and low level of injury reporting shows the need for regular continuing education on this subject.

P2018 Evaluation of the Roche Cobas HCV immunoassay

J.L.A.N. Murk*, A.M. Simoons-Smith, P.H.M. Savelkoul, M. Bosschietter-Lust, C.W. Ang (Amsterdam, Almere, NL)

Objectives: The low prevalence of Hepatitis C virus (HCV) in developed countries places large demands on the specificity of immunoassays detecting antibodies to the HCV. Frequent false-positive test results have necessitated costly and time consuming confirmatory tests. We have evaluated the performance of the new HCV immunoassay for the Roche Cobas e411 automated analyzer and compared it with the HCV immunoassay version 3.0 from Abbott for the AxSYM automated analyzer.

Methods: 917 human sera, submitted to our laboratory as part of the routine diagnostic process for HCV testing, were analyzed with the immunoassays for the Cobas and AxSYM. 437 of these sera were tested in parallel between September and December 2008.

480 sera had been submitted previously (retrospective samples) and already been tested in the AxSYM. Anti-HCV reactivity was confirmed by immunoblot or additional testing for HCV RNA.

Results: Of the 917 sera 68 tested positive in the AxSYM; 33 of these were immunoblot or HCV-PCR positive and 7 sera had equivocal immunoblots. The remaining 27/68 (40%) of initially reactive samples were regarded as false-positive. The specificity of the AxSYM HCV assay was therefore (848/876=) 96.8%. Out of 917 sera, the Cobas tested 46 sera positive; 33 were confirmed and 7 had equivocal immunoblots. Hence, 5/45 (11%) of the initially reactive samples could not be confirmed and were regarded as false-positive. The specificity of the Roche assay was (871/876=) 99.3%. Both AsXYM and Cobas HCV immunoassay detected all 33 confirmed cases of HCV infection.

Conclusion: The specificity of the new Roche HCV assay was higher than the Abbott HCV immunoassay and reduced the amount of confirmatory tests with about one third, although the difference may have been caused by selection of samples initially reactive on the AxSYM.

Further parallel studies with larger numbers of samples from patients who are HCV positive are required to solve this issue.

P2019 Hepatitis C – an opportunistic HIV coinfection: inhibitors of NTPase/helicase activity as potential antivirals

A. Baier*, R.S. Hoosmane (Lublin, PL; Baltimore, US)

Objectives: The end-stage liver diseases caused by hepatitis viral infection is one of the major causes of death (>50%) in HIV patients. HCV has lately taken the centre stage, and is ringing alarm bells in the AIDS research community for many reasons, including less effective HAART therapy. The protease inhibitors used in the HAART therapy exert a significant degree of extra strain on the liver that is already stressed by HCV. The HCV infection is in turn believed to stimulate the HIV activity. The approved anti-HCV therapy was shown to decrease the potency of anti-HIV therapy. For all these reasons, mutually compatible anti-HCV and anti-HIV drugs are urgently needed to combat HCV co-infection in HIV patients.

Between the structural and non-structural (NS) proteins encoded by the viral genome of HCV the NS3 protein appears to be one of the
most promising targets for antiviral agents because of the multiple enzymatic activities (serine protease and nucleoside triphosphatase (NTPase)/helicase) associated with this protein. Our experience with some inhibitors of the helicase activity reported previously shows that the inhibition of the enzyme may lead to reduction of the replication of the virus.

**Methods:** Recombinant HCV NTPase/helicase was purified to homogeneity from *E. coli*. Inhibition studies were performed with radioactively labelled DNA or RNA substrate.

**Results:** Previous studies performed by us indicated that the extension of the side-chain attached at the 6-position of the heterocyclic ring of the ring-expanded nucleoside (REN) analog results in inhibitory activity of NTPase/helicase. Here we present new nucleoside analogs as potent HCV NTPase/helicase inhibitors with IC50 values in low micromolar range. The nucleoside analogs that we screened may act through binding to NTPase/helicase and cause inhibition (and in some cases activation) of unwinding activity.

**Conclusions:** Since there is a close correlation between the inhibition of the helicase activity in vitro and reduction of the virus replication in vivo the compounds based on the structure of nucleosides may represent a therapeutic concept against HCV. The aim of successful therapy against HCV/HIV coinfection should be the design of compounds which exhibit dual anti-HCV and anti-HIV activities in vitro with little toxicity to the host cell lines.

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**P2020**

**Evaluation of the results of the SEIMC External Quality Control Programme for HCV viral load**

N. Orta Mira, R. Guna Serrano, M. Osies, J.L. Pérez*, C. Gimeno Cardona (Gandia, Valencia, Madrid, Palma de Mallorca, ES)

**Objective:** To analyze the results obtained in two consecutive QC controls (years 2006 and 2007) for HCV viral load (VL) launched by the Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC) External QC Programme.

**Methods:** In 2006, two plasma standards (HCV-1/06 and HCV-2/06) for the HCV VL determination by the method currently used in each laboratory were sent to 65 participants. In 2007, two different standards (HCV-1/07 and HCV-2/07) were sent to 77 participants. Standards were made after diluting plasma drawn from unique HCV-infected patients with HCV plasma from seronegative donors. Standards HCV-1/06 and HCV-1/07 had a high HCV RNA content, in opposite to HCV-2/06 and HCV-2/07. Reference VL values for each standard (mean of 3 determinations) were determined by laboratories selected by the QC Programme for each method. A confidence interval of mean±1.96 SD for the log10VL was acceptable.

Results: Effective participation rate was similar in both years (90.8%). Real time PCR was the method used for the majority of the participants (Fig.1). Reported results from participants were compared with those of the reference laboratories (same method). Acceptable results range from 87.2% (HCV-1/06) to 98.2% (HCV-2/07) for laboratories using TaqMan® Roche®. These figures were 66.7% (HCV-2/07) and 88.9% (HCV-2/06) for Cobas Amplicor Roche® and 83.3% for Abbott real time method; although these percentages should be taken prudently because of the limited number of participants by the former techniques. The same seems to apply for the bDNA Versant Siemens® methodology, but the number of participants made analysis unfeasible.

Conclusions: a) there is an increasing number of participants in the QC Programme, being similar the effective participation rate within years; b) real time PCR was the most frequent technique used; c) although the majority of the participants obtained acceptable results, the Taqman Roche® method seemed to perform better; d) external intercomparative surveys are a useful tool for quality improvement in the microbiology laboratories.

**P2021**

**Routine twenty-four mini-pool HCV RNA screening for the diagnosis of early hepatitis C virus infection in non-transfusion setting**

T. Mocnik*, K. Seme, M. Poljak (Ljubljana, SI)

**Objectives:** Because of the period of up to 3 months between hepatitis C virus (HCV) infection and seroconversion, serological assays are not very efficient for diagnosis of the early stages of HCV infection. Since HCV RNA appears in blood as early as 2 weeks after infection, the detection of HCV RNA can substantially shorten the diagnostic window, which is particularly important in blood donors and in different high risk populations. We have prospectively evaluated the usefulness of 24 mini-pool HCV RNA screening in a routine diagnostic laboratory for viral hepatitis, where more than half of newly diagnosed hepatitis C patients are intravenous drug users (IVDU).

**Methods:** A total of 15,048 anti-HCV negative samples collected between 1 June 2004 and 31 December 2008 were included in the study. A total of 627 mini-pools were tested using an automated commercial PCR assay for qualitative detection of HCV RNA, with lower limit of detection of 50 IU/ml. HCV RNA positive pools were split for further testing by the same assay. Immediately after recognition of an anti-HCV negative/HCV RNA positive sample responsible physician was informed and asked for follow-up samples.

**Results:** 30 (0.2%) anti-HCV negative/HCV RNA positive samples obtained from 23 patients (14 male, 9 female, all IVDU) were detected. 21 patients responded to invitation for follow-up testing. 15, 5 and 1 patient seroconverted in the first, second and third follow-up sample, respectively. The interval between the first HCV RNA positive/anti-HCV negative sample and the first anti-HCV positive sample was between 19 and 154 days. Considering viral loads measured in anti-HCV negative/HCV RNA positive samples, even 48 mini-pool strategy could be used without reducing the sensitivity. The costs of detecting a single anti-HCV negative/HCV RNA positive sample and a single viremic seronegative patient using this strategy were estimated to be around €903 and €1178, respectively.

**Conclusion:** Combined screening using anti-HCV and 24 mini-pool HCV RNA testing can be useful and cost effective outside a blood transfusion setting, at least in laboratories at which significant proportion of tested patients belongs to high-risk populations.

**P2022**

**Expression of interferon-induced microRNAs in patients with chronic hepatitis C virus treated with pegylated interferon alpha**

C. Scagnoli*, P. Zingariello, C. Selvaggi, A. Zoccoli, J. Vecchiet, S. Cisciti, E. Pezzigallo, G. Antonelli (Rome, Chieti, IT)

**Objectives:** In order to further elucidate the determinants of response to interferon (IFN) therapy in patients with chronic hepatitis C, a gene expression analysis of cellular microRNAs (miRNAs), which has been previously reported to be involved in IFN-mediated antiviral activity against hepatitis C virus (HCV), has been performed.

**Methods:** The expression of several miRNAs (mir-1, -30, -128, -196, -296) was retrospectively measured in peripheral blood of mononuclear cells (PBMC) derived from 12 patients with HCV before and after 12 hours from the first injection of IFN. Gene expression
Acute hepatitis C and nosocomial transmission of hepatitis C virus: an emergent threat in the hospital setting?

G. Corti*, F. Baragli, A. Cavallo, D. Bartolozzi, S. Ambu, F. Leoncini, A. Bartoloni (Florence, IT)

Objective: Symptomatic acute hepatitis C (AHC) is rarely identified in the clinical practice, it is frequently followed by the spontaneous resolution (SR) of hepatitis C virus (HCV) infection without evolution into chronicity, and it generally responds to standard antiviral therapy better than chronic hepatitis C does. We prospectively followed all consecutive AHC cases we observed in the inpatient/outpatient services of our hospital units during the last three-year period, in particular as regards: 1) risk factors; 2) clinical outcome; 3) efficacy of treatment, if any.

Methods: Between 1st January 2005 and 31st December 2007, we diagnosed symptomatic AHC in 13 males, median age 54 years; main demographic characteristics are shown in the table. At the 12-week follow-up, we began pegylated interferon (PegIFN) + ribavirin in patients who had detectable plasma HCV RNA as measured by PCR (Cobas Amplicor Monitor®, Roche, in copies/mL up to October 2005; TaqMan® RT-PCR, Roche, in IU/mL from November 2005), whereas the PCR-negative patients were followed up at least for 24 weeks, after which they were considered as having SR of their AHC if still PCR-negative.

Results: A total of 13 patients met the criteria of acute hepatitis C infection. Of those, 11 went on to complete treatment, while two were lost to follow-up. Six patients were SR-positive (46.2%), three were treatment failures (23.1%), and four patients never cleared viremia (30.8%). The treatment failures were due to virological non-response (VN). One patient died of causes unrelated to hepatitis C infection.

Conclusion: In our experience with AHC, PegIFN + ribavirin was not as effective as in chronic hepatitis C patients. The reasons for this could be related to the short follow-up period and the small sample size.

<table>
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<tr>
<th>Age Risk factors</th>
<th>Genotype</th>
<th>Peak PCR</th>
<th>Peak ALT</th>
<th>Therapy</th>
<th>Outcome</th>
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*ERCP=endoscopic retrograde cholangiopancreatography; ALT= Alanine aminotransferase; SR= spontaneous resolution.

Conclusions: Our study, albeit carried out on a small number of patients, confirms the results of recently published papers on some features of AHC, in particular: 1) the increasing impact of nosocomial HCV acquisition resulting from unsafe hospital practices and contaminated equipment; 2) the frequent SR of symptomatic AHC. We stress the need of: 1) strict adherence to universal precautions in order to minimise the risk of nosocomial HCV transmission; 2) wait for the first 12 weeks after acute infection in order to observe the possible SR of AHC.

Transversal study in a group of Spanish HIV/HCV co-infected patients with non-treated chronic hepatitis C: epidemiological study, prevalence and grade of hepatic fibrosis

E. Valencia*, V. Moreno, L. Martín-Carbonero, J. González Lahoz (Madrid, ES)

Objectives: 1) To analyse hepatic fibrosis using non-invasive methods in a group of HIV and non-treated HCV infected patients and 2) To determine the factors that influence on fibrosis development: sex, alcohol and illegal drugs abuse, hepatitis C genotype, co-infection with HBV and HDV, HIV and HCV viral load, CD4+ cells and HAART use. It was also analysed why patients were not receiving treatment for HCV infection.

Methods: This is an observational and transversal study. Patients are a subgroup of the multicentric study GRAPHICO and all had active HCV infection without treatment. Hepatic fibrosis was measured by Fibroscan and APRI/Forns index. Statistical analysis was done by SPSS 13.0.

Results: 102 patients were included and most of them were male (71%), 93 (91%) had been IVDA (only 4 active drug users), 22.5% had been heavy alcohol drinkers, 81% were smokers and 7% consumed cannabis. Genotype 1 was the most frequent (61%), 7 were co-infected with HBV and 3 with HDV. Causes for no treatment were: patient rejection (52%), previous fracases (22%) and contraindicated therapy and/or toxicity (25.5%). Mean HCV viral load was 1.2×10⁶ copies/ml and only 28 (27.5%) had detectable HIV viral load. Most subjects were receiving HAART (89%) and mean CD4 cells was 479 mm³. The CD4+ nadir was 220 per mm³. Mean time of HCV infection was 12 years. Fibrosis was detected by APRI/Forns in 21 patients (20.6%) and Fibroscan was realised in 78 (76.5%) showing F0-F1 (<7 kPa) in 33%, F2 (7.1–9.4 kPa) in 20%, F3 (9.5–12.2 kPa) in 11% and F4 (>12 kPa) in 35%. As compared with those without significant fibrosis, absolute, percentage and nadir of CD4+ cells, platelets count, cholesterol level and prothrombin activity were lower in patients with significant fibrosis (p < 0.05). Similarly, genotype 1, male sex, alcohol intake, tobacco and cannabis consumption and HbsAg+ were more frequent in subjects with significant fibrosis (p < 0.05). There was a direct correlation of fibrosis grade by transient elastography and APRI/Forns index.

Conclusions: 1) Patients co-infected with HIV and HCV who are not receiving treatment for HCV have more hepatic fibrosis if they are men, if they are co-infected with HBV, if they have genotype 1 and if they are smokers, heavy alcohol drinkers and cannabis consumers. 2) Fibrosis is more significant in those with lower absolute, percentage and nadir of CD4+ cells. 3) Fibroscan and APRI/Forns index are similarly for determining hepatic fibrosis using non-invasive methods.

Genetic diversity of hepatitis C virus among Bulgarian injecting drug users with hepatitis C

P. Teoharov*, I. Pavlov (Sofia, BG)

Objective: To assess the genotype diversity of hepatitis C virus (HCV) among Bulgarian injecting drug users with hepatitis C.

Methods: Serum samples from 147 anti-HCV positive injecting drug users were tested by qualitative RT-PCR assay AMPLICOR HCV virus (HCV) Test, version 2.0 (Roche Molecular Systems, Inc, Branchburg, NJ, USA). Commercially available enzyme immunoassay ETI-AB-HCVK-4 (Dia Sorin, S.p.A. Italy) was used to detect anti-HCV antibody. Genotyping of HCV RNA obtained from serum samples was performed using Versant HCV Genotype Assay (LiPa) – Bayer HealthCare LLC, Belgium.

Results: Since January till November 2008 a total of 147 anti-HCV positive serum samples from Bulgarian injecting drug users, were tested...
Development of a low-cost approach for quantitation and genotyping of hepatitis C virus


Objectives: Concentration of HCV RNA in plasma and its genotype determines the chance of therapeutic response and duration of treatment. Our goal was to develop a low-cost method for simultaneous quantitation and genotyping of HCV RNA.

Methods: A Real-time RT-PCR assay for quantitation of HCV RNA was developed. A pair of primers and hybridisation probes was selected that were specific for recognition of 5′UTR in HCV genome. Calculation of HCV viral load was based on an external standard curve using standard RNA calibrated with NIBSC standard panel. A genotyping method was developed using amplification products obtained from the HCV RNA quantitation. Determination of HCV genotype was performed by melting curve analysis using a pair of hybridisation probes.

Results: Our assay had a sensitivity of 50 IU/ml, with a dynamic range of detection between 103 and 106 IU/ml. The CV of threshold cycle values in intra- and inter-assay were less than 1.77% and 3.40% respectively. Parallel analysis by this new assay and Real-time RT-PCR commercial kit on 56 clinical samples in different times of treatment with IFN-α2a and ribavirin, showed a good correlation (Before treatment, $R^2 = 0.934$; $p < 0.05$), (1 month after treatment, $R^2 = 0.992$; $p < 0.05$), (3 months after treatment, $R^2 = 0.992$; $p < 0.05$). Genotypes were determined by melting curve analysis and compared to the results of PCR-RFLP with good correlation (Kappa = $0.873$, $P < 0.001$).

Conclusion: Our method has a good sensitivity and specificity for detection, quantitation and genotyping of HCV RNA within approximately 2 hours. They can be a good replacement for commercial kits especially for clinical evaluation of therapy.

Oxidative stress and antioxidant defence in patients with chronic hepatitis B and C


Objectives: Oxidative stress is defined as a disturbance of balance between free oxidative radicals and antioxidant substances. This study investigated the in patients oxidative stress with chronic hepatitis B and C.

Methods: Ninety eight patients with chronic viral hepatitis admitted to Department of the Infectious Diseases and Clinical Microbiology of Medical Faculty of Ondokuz Mayis University were enrolled into study. Twenty healthy persons were included as control group. Study group were divided into four groups as healthy controls (group 1), chronic hepatitis B (group 2), chronic hepatitis C (group 3) and inactive hepatitis B carriers (group 4). Antioxidant status of plasma, including glutathione, glutathione peroxidase, vitamin E and vitamin C levels, were measured. Carbonyl and lipid peroxidation levels were measured as parameters of oxidative stress.

Results: Glutathione, glutathione peroxidase, vitamin E and vitamin C levels were found to be significantly decreased in chronic hepatitis B group when compared with control group (9.5 vs 13.6, $p < 0.05$; 22.98 vs 32.4, $p < 0.05$; 15.1 vs 16.4, $p < 0.05$; 12.9 vs 18.4, $p < 0.05$). Carbonyl and lipid peroxidation levels were significantly increased in chronic hepatitis B group than those of controls (0.7 vs 0.5, $p < 0.05$; 2 vs 0.7, $p < 0.05$). Similarly, glutathione, glutathione peroxidase, vitamin E and vitamin C levels were found to be significantly decreased in chronic hepatitis C group when compared with control group (9.2 vs 13.8, $p < 0.05$; 17.7 vs 32.4, $p < 0.05$; 14.7 vs 16.4, $p < 0.05$; 11.1 vs 18.4, $p < 0.05$), and carbonyl and lipid peroxidation levels were significantly increased in chronic hepatitis C group as compared with controls (0.8 vs 0.5, $p < 0.05$; 1.8 vs 0.7, $p < 0.05$). However, whereas glutathione and carbonyl level correlation with HBV DNA levels were mild to moderate (glutathione vs HBV DNA, $r = 0.288$, $p < 0.05$; carbonyl vs HBV DNA, $r = 0.317$, $p < 0.05$), lipid peroxidation level were strongly related with HBV DNA levels in chronic hepatitis B ($r = 0.545$, $p < 0.05$). It was not determined any correlation between HCV RNA and oxidative or antioxidative parameters.

Conclusion: In conclusion, there was decrease at the level of protective antioxidative parameters, where as there was increase at the level oxidative parameters in hepatitis B and C patients.

APRI Index, HCV genotype and HCV RNA as predictors of early viral response in hepatitis C patients

J. Mata-Marin*, J. Fuentes-Alleen, J. Gaytan-Martinez, A. Chaparro-Sánchez, C. Arroyo-Anduiza, B. Manjarrez-Telles (Mexico City, MD)

Background: Absence of therapeutic response in hepatitis C patients is multifactorial; genotype 1, viral load major than 800,000 IU/ml, and APRI index of hepatic fibrosis major than 1.2 are associated to an unfavourable response.

Methods: We prospectively checked patients with diagnosis of hepatitis C since July 2006 to February 2008, who were evaluated to start therapy with interferon α2b pegylated and ribavirin and reviewed retrospectively clinical charts of patients with the same diagnosis from January 2004 to June 2006. The treatment with Interferon α2b pegylated 1.5 μg/kg/weeks and ribavirin was adjusted (>75kg: 1200 and <75kg: 1000mg). Patients were allocated in one of two groups: Group 1 included patients with hepatitis C with early viral response (EVR) and group 2 patients without EVR. We identify any clinical and/or biochemical variable potentially predictive of the response.

Results: During the study, 80 patients were analyzed, 45 in retrospective way and 25 in prospective way. The mean (+SD) age of these patients was 42.9±12 years. 55 (68.8%) were genotype 1 and 25 (31.3%) were genotype 2 or 3. Variables associated with absence of EVR were genotype 1 (OR 0.28 IC 95% 0.08–0.94; $p = 0.034$) and the combination of the factors genotype 1, APRI index >1.2 and HCV RNA >800,000 IU/ml (OR 0.162 IC 95% 0.02–0.89; $p = 0.021$). After adjustment in a logistic regression model, only the factor genotype 1 remains significant.

The efficacy and adverse events of standard interferon (alpha 2a or 2b) plus ribavirin versus pegylated interferon (alpha 2a or 2b) plus ribavirin

H. Gedik*, M. Yuhyaoglu, M. Fincanci (Istanbul, TR)

Objectives: In this study the efficacy and adverse events of standard interferon alpha 2a (or 2b) plus ribavirin versus peginterferon alpha 2a (or 2b) plus ribavirin was evaluated in patients with chronic hepatitis C.

Methods: A total of 98 naive patients with biopsy proven chronic hepatitis, elevated ALT levels, and positive HCV-RNA were enrolled. Fifty-six patients received standard interferon α2a or 2b (3 MIU tiw) plus ribavirin (1000–1200mg qd) for 52 weeks (Group A) and 42 patients received peginterferon α2b (1.5 μg/kg subcutaneously weekly) or Pegylated interferon α2a (140 μg or 180 μg subcutaneously weekly) plus ribavirin (1000–1200 mg qd) for 52 weeks (Group B).
Emerging or re-emerging infections

**P2030** Evaluation of the anthrax cases who were admitted to our hospital in 2008
K. Ozden*, Z. Ozbek, A. Kadanali, S. Erol, M. Parlak (Erzurum, TR)

**Objectives:** The study was conducted to evaluate characteristics of the cases with anthrax, which is an endemic zoonosis in northeastern region of Turkey.

**Methods:** The cases who were admitted to our hospital with the suspicion of anthrax in 2008 were included in the study. After first evaluation, the cases with confirmed diagnosis of anthrax were subsequently evaluated with a standard questionnaire inquiring demographic characteristics, risk factors and clinical data of the cases.

**Results:** A total of 27 cases, including 18 male (66.7%) and 9 female (33.3%), were diagnosed as anthrax in 2008. All cases had a history of exposure to sick animals, and were living rural areas. Exposure types were skinning, butchering a sick animal and handling and eating contaminated meat. The animals which were exposed to were sheep (n=4, 15%) and cattle (n=23, 85%). All the cases were cutaneous anthrax. Lesions were mostly located in wrist and arms (n=12, 44.4%) followed by hands and fingers (n=11, 40.7%) and eyelid and faces (n=4, 14.8%). Twenty six cases were diagnosed between August and October.

**Conclusions:** This study shows that anthrax still remains as an important health-problem in Turkey. It was common in males, mostly resulted from cattle exposure, had seasonal characteristic and mostly was seen in cutaneous form. Good surveillance, decontamination and disinfection procedures, and education are mandatory to reduce the incidence of anthrax and also employees should be educated about the disease to reduce the risk for disease. Controlling the disease in humans ultimately depends on controlling it in animals by effective surveillance and immunisation.

**P2031** Clinical features and epidemiology of leptospirosis in Spain

Leptospirosis is a worldwide distributed zoonosis, caused by infection with pathogenic *Leptospira* species. We analyzed the cases between January 1994 and September 2008. Clinical presentation, laboratory data, treatment, evolution and complications were evaluated.

34 patients (25 males and 9 females) were diagnosed of leptospirosis. The median age was 41 years. Confirmation of the diagnosis was made in 32 cases by ELISA and in two cases by detection of Leptospiral DNA in urine (IgM initially negative with posterior sero-conversion). The risks factors of exposure were assessed:10 patients were rice farmers, 6 patients have been in contact with irrigation ditches, 2 were bricklayers and in two cases an international travel was associated. The more frequent symptoms were: fever (100%), artrromyalgia (56%), dispnea (32%), Shock (20%), Abdominal pain(36%), neurologic involvement(15%) and jaundice(24%). The laboratory data showed: 16 patients renal impairment(43% precise dialysis), 70% rises in transaminase levels and 11% elevated levels of alkaline phosphatase. 24% presented with a total bilirubin count more than 10mg/dL. Elevation of CPK occurred in 25 patients and the levels of laddomiolisis were correlated with renal insufficiency and worse evolution.48% of the cases presented with thrombopenia (17% the thrombopenia less than 20,000 platelets) that was associated with low Quick index in 16%. 55% of the patients presented with non significative alterations of the urine sediment. Lumbar puncture was performed in 5 patients being suggestive in all cases of lymphocytic meningitis and the culture of the cerebrospinal fluid in Fletcher medium was negative. Two patients suffered pulmonary involvement: One suffered pleural effusion and one patient presented with alveolar haemorrhage. Ten patients suffered severe complications, with intensive care hospitalisation. The mortality rate was 20%. The more common antibiotic used was Doxycycline (11 cases), Penicillin (5 cases), cephalosporines (8 cases), quinolones (4 cases) and other combinations that included carbapenems.

Our area is endemic in *Leptospira* due to farmers and rice-workers. Renal deterioration and alveolar haemorrhage were severe complications correlated with intensive care hospitalisation and death. Due to the fastidious growing of *Leptospira* spp., diagnosis has traditionally established in our hospital by serology; this study emphasizes the pivotal role that molecular biology can play now in order to get an early diagnosis.

**P2032** In vitro activity of antimicrobials in combination against clinical strains of extreme drug-resistant *Acinetobacter baumannii* resistant to all antibiotics including polymyxin B in Singapore
T.P. Lim*, W. Lee, T.Y. Tan, S. Sashihala, T.T. Tan, L.Y. Hsu, A. Kwa (Singapore, SG)

**Objectives:** We have used polymyxins since 1990s in Singapore. Emergence of extreme-drug resistant (XDR) *Acinetobacter baumannii* (AB) infection resistant to all available antibiotics including polymyxins have finally occurred in an immunocompromised patient with haematological malignancy after weeks of polymyxin B(PB) therapy. Combination therapy may be the only viable option until new antibiotics become available. We assess the in vitro activity of various antimicrobials and elucidate the most effective combination therapy against these XDR AB. **Methods:** 2 isolates (AB 1 and AB 2) from the patient was collected from blood samples on different days. MICs were determined according to a modified CLSI broth-dilution method. Time-kill studies (TKS) were performed with approximately 10⁶ CFU/ml at baseline with the maximum, clinically achievable, unbound concentration (mg/L) of PB (2), (R)rifampicin (2), (M)meropenem (64), (C)cefepime (300) and (T)tigecycline (2) alone and in combination against the 2 isolates.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>XDR AB 1</th>
<th>XDR AB 2</th>
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<tbody>
<tr>
<td>Ampicillin/Sulbactam</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Ciprofloxacix</td>
<td>≥16</td>
<td>≥16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥64</td>
<td>≥64</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≥64</td>
<td>32</td>
</tr>
<tr>
<td>Meropenem</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≥128</td>
<td>64</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>≥256</td>
<td>≥256</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>≥128</td>
<td>≥128</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≥2048</td>
<td>≥2048</td>
</tr>
<tr>
<td>Cefepime</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>2</td>
<td>4</td>
</tr>
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</table>
Results: MICs to various antibiotics are shown in Table 1. Both isolates were resistant to all antibiotics including PB (MICs 32–64 mg/L). In single drug TKS, all drugs were bacteriostatic at 24 h except for PB and T where the reduction in bacterial burden could hardly be seen against AB 1. Against AB 2, all drugs were bacteriostatic at 24 h except for PB where the reduction in bacterial burden could hardly be seen. In combination TKS, no combination was synergistic against AB 1 whereas RP, MP and CP were synergistic against AB 2. They were bactericidal at 8 h and exhibited sustained kill till 24 h.

Conclusions: Clinical isolates of AB resistant to PB is also resistant to all major antibiotic classes with no compromise in biofitness; in contrary to previous reports that illustrate PB resistant AB with a substantial deficit in biofitness in vitro. We had shown that RP, MP and CP may be potential antibiotic combinations as pre-emptive therapy for XDR AB infections and warrants further investigations.

**P2033 Effective antimicrobials in combination against extreme drug-resistant Pseudomonas aeruginosa with decreasing susceptibilities to polymyxin B in Singapore**

T.P. Lim, W. Lee, T.Y. Tan, S. Siahuala, T.T. Tan, L.Y. Hsu, A. Kwast (Singapore, SG)

Objective: Emergence of extreme drug-resistant (XDR) Pseudomonas aeruginosa (PA) infections in immunocompromised hosts with decreasing susceptibilities (DS) to Polymyxin B (PB) have occurred in Singapore. Combination therapy may be the only viable option until new antibiotics become available. The objective of this study is to assess the in vitro activity of various antimicrobials against XDR PA isolated from our local hospitals.

Methods: Three clinical XDR PA isolates with MIC 4–8 to polymyxin B collected from each public hospital (SGH, NUH, TTSH) in Singapore were studied. Time-kill studies (TKS) were performed with approximately 1 x 10³ to 5 x 10⁵ CFU/ml at baseline in clinically achievable unbound concentrations of PB (2 mg/L), levofloxacin (24 mg/L), rifampicin (R) (2 mg/L), amikacin (A) (80 mg/L) and meropenem (M) (64 mg/L) alone and in combination. TKS screening were performed with the above combinations elucidated against additional 12 clinical XDR PA isolates from the same hospitals.

Results: In TKS, a sustained killing effect of >99% (>2 log kill) from baseline inoculum at 24 h was only seen with at least 3-drug combination AMP (SGH), LRP (NUH), ALRP (TTSH), AMRP (TTSH) and AMRP (TTSH). No regrowth was observed at 24 h. A sustained killing effect of >99% by AMP was observed against 7 out of 12 screening isolates. A sustained killing effect of >99% by LRP was observed against 7/12 isolates. Of these 7/12 isolates (sustained killing seen with LRP), 3/12 isolates did not achieve sustained killing with AMP. ALRP, AMMP and AMRP when used in TKS screening for 2 isolates (did not achieve >99% killing effect with AMP or LRP), achieved >99% killing effect with 0/2, 0/2, and 1/2 isolates respectively.

Conclusion: AMP, LRP and AMRP achieved sustained killing effect of >99% in 11/12 screening isolates. Sustained killing effect of >99% is important in eradicating XDR PA infection, especially in immunocompromised hosts. These findings demonstrate that in vitro synergy of at least 3-antibiotic combinations in XDR PA were required to eradicate this XDR PA which may be strain dependant. It may guide us in making useful predictions on choosing a pre-emptive therapy for XDR PA infections with decreasing susceptibility to Polymyxins, however, it warrants further investigations.

**P2034 Investigation of N. cyriacigeorgica and N. abscessus infections: a recent experience of 6 cases in Greece**


Objectives: Nocardia infections are of great importance, especially in immunocompromised patients. However, identification of strains in species level is difficult in routine microbiology. The aim of this study was to further investigate the cases of Nocardia infection occurred in our Hospital, within 2008, in the terms of isolate identification and antibiotic susceptibility in bacterial burden could hardly be seen against AB 1. Against AB 2, all drugs were bacteriostatic at 24 h except for PB where the reduction in bacterial burden could hardly be seen. In combination TKS, no combination was synergistic against AB 1 whereas RP, MP and CP were synergistic against AB 2. They were bactericidal at 8 h and exhibited sustained kill till 24 h.

Conclusions: Clinical isolates of AB resistant to PB is also resistant to all major antibiotic classes with no compromise in biofitness; in contrary to previous reports that illustrate PB resistant AB with a substantial deficit in biofitness in vitro. We had shown that RP, MP and CP may be potential antibiotic combinations as pre-emptive therapy for XDR AB infections and warrants further investigations.

**P2035 Pertussis remains a health problem**

D. Karabasoglou, E. Bakali, P. Siatos, A. Kafantzi, E. Dima, A. Kansozidou* (Thessaloniki, GR)

Introduction: Pertussis is a highly contagious disease of childhood. Clinical diagnosis is confirmed by laboratory tests, especially by the detection of IgM, IgG and IgA specific antibodies in the serum of patients. The immunity from infection or vaccination lasts only a few years. Therefore, an increase on adults vulnerable to the infection is observed.

Aim: The aim of this report is the study of pertussis cases that were recorded during the decade of 1999–2008, the estimation of the laboratory findings and the frequency of the disease according to age.

Material and Methods: A total of 373 subjects (173 males and 200 females) aged 17 days to 65 years old were studied and serum samples collected from them were analyzed. These subjects were divided into three groups. First group: 283 children who were hospitalised with the clinical diagnosis of pertussis. Second group: 57 house-hold contacts of 57 children with laboratory confirmed pertussis. Third group: 33 adults who suffered from chronic cough. All serum samples were tested for B. pertussis specific IgM, IgA and IgG antibodies using ELISA. The diagnosis of pertussis was based on positive titers of IgM or/and IgG and IgA specific antibodies. Also, a significant increase in IgG specific antibodies between paired samples and an epidemiological linkage with a confirmed case were estimated.

Results: In 245 subjects the disease was confirmed by laboratory findings. In 166 children of the first group a positive titer of IgM or/and IgG and IgA antibodies was detected in single serum or paired serum samples. A close contact with pertussis was found in 35 children of the first group. The diagnosis of the disease in 11 children was established by epidemiological linkage with a confirmed pertussis case from the family environment. The prevalence of the disease was higher in children older than eight years old (85%) than in children younger than four years old.
Pigs as a source for toxigenic Corynebacterium ulcerans in diphtheria-like disease
(Oberschleissheim, Erlangen, Coburg, DE)

Objectives: Toxigenic Corynebacterium ulcerans may cause a zoonotic infection similar to C. diphtheriae-caused diphtheria. Previously, mainly dairy cattle were described as C. ulcerans reservoirs, while recent publications suggest pet dogs and pet cats as carriers, the latter often affected by binasal discharge. Here, we report the first case of severe C. ulcerans diphtheria-like disease after pig contact in a previously healthy farmer presenting with severe diphtheria-like illness including polyneuropathy and cardiomyopathy.

Methods: Species identification of C. ulcerans was achieved by biochemical differentiation (API Coryne), rpoB sequencing and MALDI-TOF analysis. Toxigenicity of the strain was verified using a C. diphtheriae tox-PCR, a C. ulcerans tox-specific PCR and the Elek test as described previously. An outbreak investigation involving both the patient’s family and their farm animals (19 pigs and one dog) was started.

Results: Pharyngeal swabs of 3 family members, 19 pigs and the farm dog were obtained and analysed for C. ulcerans. While all family members and the dog were C. ulcerans-negative, one of the 19 asymptomatic pigs harboured a toxigenic C. ulcerans strain. Sequencing of rpoB and tox revealed 100% homology between the human and the pig strain. Ribotyping confirmed this result suggesting the identity of both strains.

Conclusion: To our knowledge, this is the first case of proven transmission of a toxigenic C. ulcerans strain between a livestock animal and a human. Moreover, harbouring of toxigenic C. ulcerans has previously not been reported in pigs. As the handling of C. ulcerans-infected pigs might lead to diphtheria-like illnesses, studies on toxigenic C. ulcerans carriage among pigs are certainly needed.

Toxigenic Corynebacterium ulcerans may cause a zoonotic infection similar to C. diphtheriae-caused diphtheria. Previously, mainly dairy cattle were described as C. ulcerans reservoirs, while recent publications suggest pet dogs and pet cats as carriers, the latter often affected by binasal discharge. Here, we report the first case of severe C. ulcerans diphtheria-like disease after pig contact.

Detection and enumeration of Clostridium difficile in retail meat
J.S. Weese*, B. Avcory, J. Rousseau, R. Reid-Smith (Guelph, CA)

Objectives: Community-associated C. difficile infection appears to be an increasing problem and concern has been expressed about food as a source of infection. While studies have identified C. difficile in retail meat, the level of contamination has not been reported. The objectives of this study were to determine the prevalence and concentration of C. difficile spores in retail meat and to characterise recovered isolates.

Methods: Ground beef and ground pork were purchased from retail outlets in 4 Canadian provinces. Broth enrichment using a rinse of ground meat into CDMN broth with 0.1% sodium taurocholate and inoculation onto CDMN agar was used for qualitative analysis. Quantitative testing was performed using serial 10-fold dilutions of the rinses and inoculation onto CDMN agar. Ribotyping, toxotyping and toxin gene PCR were performed on isolates.

Results: C. difficile was isolated from 27/230 (12%) samples overall, 14/115 (12%) ground beef and 14/115 (12%) ground pork (P=1.0). For ground beef, 10/14 (69%) were positive on enrichment culture only while 2/14 (14%) were positive on both enrichment and direct culture and 2/14 (14%) were positive on direct culture only. Of the 4 ground beef samples that were positive on direct culture, 20 spores/g were present in two while 120 and 240 spores/g were present in one each. For ground pork, 10/14 (71%) were positive on enrichment culture only while 2/14 (14%) were positive on both enrichment and direct culture and 2/14 (14%) were positive on direct culture only. Of the 4 ground pork samples that were positive on direct culture, 20 spores/g were present in three while 60 spores/g were present in one. All samples that were positive on direct but not enrichment culture only contained 20 spores/g. Typing data are presented in the table.

Discussion: This is the first study to quantify C. difficile contamination in retail meat and the finding of low levels may be important. While the infectious dose for C. difficile is not known and may be variable between individuals, it is plausible that low numbers of spores are less relevant than larger numbers. Yet, this low level contamination should not be dismissed. The predominance of toxin variant strains in not surprising, and the finding on ribotype 078 which has been associated with CA-CDI and ribotype 027, an important epidemic strain, raise concerns. Further study of food as a source of infection in warranted.

Clinical characteristics of infections with Clostridium difficile ribotype 027 versus other ribotypes: data from prospective surveillance in Belgium
M-L. Lambert*, J. Van Broeck, C. Fontaine, C. Pulincs, V. Acesani, M. Delmée (Brussels, BE)

Objectives: To compare clinical characteristics of patients infected with C. difficile ribotype 027, and those with other ribotypes.

Methods: We linked patient data from the prospective surveillance of C. difficile in Belgian hospitals, with typing data from the Belgian C. difficile reference laboratory. We compared CDI with, and without, ribotype 027, in terms of patient characteristics, type of infection (hospital-associated, or not), length of stay in hospital post infection, and outcome.

Results: Laboratory data were available for 980 episodes of C. difficile infections (CDI) which occurred between July 1, 2006, and June 30,
**Clostridium difficile**

Prevalence of **Clostridium difficile** in retail pork

D.S. Metcalf*, R.J. Reid-Smith, B.P. Avery, J.S. Weese (Guelph, CA)

**Objective:** Clostridium difficile has been isolated from varying percentages of healthy animals, and some strains found in food animals are those implicated in infections in humans. To date, only a few studies assessing *C. difficile* contamination of food products have been performed and few have used systematic sampling methods over broad geographic regions. The objective of this research was to determine the prevalence of *C. difficile* in Canadian retail pork products and to characterise these isolates.

**Methods:** Retail pork was collected from 4 Canadian provinces between November 2007 and May 2008. Five grams of each sample were incubated anaerobically in a *C. difficile* selective medium for 7 days and plated onto blood agar plates. The colony identities were confirmed using biochemical tests and isolates were characterised using standard typing techniques including ribotyping and toxotyping, in addition to being screened for the presence of tcdA, tcdB, binary toxin gene (cdtB), and tcdC sequence analysis.

**Results:** *C. difficile* was isolated from 1.8% (7/393) retail pork samples; 4/296 (1.4%) ground pork samples, and 3/97 (3.1%) pork chops. Five different ribotypes and 3 different toxino-types were identified. Three isolates were ribotype 027 and toxino-type III, with genes encoding toxins A, B and binary toxin, an 18bp tcdC deletion and a truncating mutation in tcdC. One strain had a different ribotype from 027 but was toxino-type III, positive for all 3 toxin genes and had the same tcdC deletion and mutation as 027. One isolate was toxino-type XXVII and possessed genes for toxins A and B and an unaltered tcdC gene, while another was a toxino-type 0 strain possessing the same toxin gene characteristics. One isolate was non toxogenic. All toxigenic strains have been found in people in Canada. There were no statistically significant associations between prevalence of *C. difficile* and province (p = 0.28) or sample type (p = 0.37).

**Conclusions:** Although the implicatios for food safety practices remain elusive, the frequency of toxigenic isolates and isolates indistinguishable from known human pathogenic strains suggests contaminated pork may be a source of *C. difficile* in humans. While the prevalence of contamination was lower here than in some other reports, further investigation of sources of contamination and clinical relevance are needed.
group housed calves initially and 46/93 (49%) later (P = 0.037). Overall, *C. difficile* was isolated from 53/193 (33%) calves initially and 88/162 (54%) 6 days later. There was no difference in the prevalence of colonisation between management types at either sampling point (P = 0.87 and 0.16 respectively). 14 of the 31 (45%) group housed calves that were positive initially were negative on the 2nd sample, as were 7 (32%) of the initially-positive individually housed calves. Virtually all calves had some degree of diarrhoea at the time of the second sampling, which is typical for this farm. The role of calves had some degree of diarrhoea at the time of the second sampling, which is typical for this farm. The role of calves housed in the study is available from France. We aimed at assessing the HEV RNA circulation of the HEV in wild boars, and, to our knowledge, no

Conclusions: The colonisation rate of veal calves in this study was high, even at the first sampling time and increased significantly shortly after arrival. The significant increase was not surprising and various factors, including stress of transportation, diet change, environmental exposure and antimicrobial administration, could be involved. Homogenous management of calves, including antimicrobial therapy, precludes evaluation of factors associated with colonisation. Further study of these calves through their lifetime on the farm and evaluation of typing data will provide additional insight into the epidemiology of *C. difficile* in veal calves.

**P2043** First detection of *Clostridium difficile* ribotype 027 in Bochum, Germany, confirmed by slpA sequencing

M. Kaase*, P. Szabados, A. Anders, T. Sáticz, G. Gatermann (Bochum, DE)

**Objectives:** Due to its increased virulence reliable detection of *C. difficile* Type 027 is necessary. In Germany *C. difficile* ribotype 027 has been described only in the south-west.

**Methods:** *C. difficile* was cultured from stool samples of hospitalised patients using selective media. *C. difficile* isolates were tested for the presence of tcdB by PCR. Susceptibility testing for erythromycin and moxifloxacin was used to screen for ribotype 027 and all isolates resistant to erythromycin or moxifloxacin were further characterised. A duplex PCR for cdtA and cdtB was performed. Isolates with positive results were typed by PCR ribotyping according to Stubbs et al. If results suggested ribotype 027 we performed slpA and tcdC sequencing to confirm the results.

**Results:** Of 130 tcdB positive *C. difficile* isolates resistant to erythromycin and moxifloxacin collected between April and December 2008 we could demonstrate the genes for the binary toxins cdtA and cdtB in 47 (36.2%) isolates. PCR ribotyping gave the same banding patterns as in a ribotype 027 reference strain in 9 isolates. All those isolates showed slpA sequences typical of ribotype 027 and deletions at position 117 and 330 to 347 of the tcdC gene. Three cases of *C. difficile* ribotype 027 occurred in the same hospital ward. The remaining cases were found in different hospitals. The mean age of cases was 68.6 years (range 12 to 90).

**Conclusion:** For the first time cases of *C. difficile* ribotype 027 could be demonstrated in our area. All isolates carried the genes for the binary toxins cdtA and cdtB and deletions in the gene for the negative regulator of toxin production tcdC.

**P2044** Leptospirosis: an emerging disease in rural areas in Greece

E. Papathimitriou*, A. Masgala, D. Sfrasar, G. Kiriazopoulos, S. Pournaras, T. Douros (Lamia, Athens, Larissa, GR)

**Objectives:** To evaluate the incidence, clinical course and outcome of leptospirosis in a tertiary care hospital in Greece.

**Methods:** All cases of leptospirosis were recorded during one year period in our hospital.

**Results:** A total of 7 cases of leptospirosis were recorded. Of these, 6 were males and 1 was female. The mean age was 54.6 years. All patients were farmers. Five of these (71.4%) presented with severe leptospirosis (Weil’s syndrome) having jaundice, renal failure, and haemorrhage. Two of the patients with severe leptospirosis experienced ARDS and intrapulmonary haemorrhage during the course of the disease, whereas the others three required renal dialysis for a time period of 1 month to one year. The rest of the patients with leptospirosis presented only with fever and thrombocytopenia while the course of the disease was uneventful. Of note, thrombocytopenia (PLT < 70,000) was present in all cases. Diagnosis was confirmed by the fourfold rise of antibodies against leptospira interrogans. The patients were treated successfully with ceftriaxone, but 3 of them experienced the Jerich Herxheimer reaction. The outcome was good in all patients.

**Conclusion:** Leptospirosis is still emerging in rural areas in Greece. Although in the literature Weil’s syndrome develops in 5–10% of infected individuals, in our study concerns the 71.4% of the patients. Thrombocytopenia, although it is usually associated with renal failure, it was present in all cases examined.

**P2045** Hepatitis E virus detection in wild boar liver samples from south-eastern France

M. Kaba, B. Ducouste, J.L. Marié, J.M. Rolain, D. Raoult, P. Colson* (Marseille, Toulon, FR)

**Objectives:** Autochthonous hepatitis E is currently considered as an emerging disease in industrialised countries. To date, the routes of transmission of hepatitis E virus (HEV) in these countries remain largely unknown. However, a growing body of data suggests that animals, especially pigs, might be reservoirs for HEV and a source for its transmission to humans. In contrast, only few data are available about the circulation of the HEV in wild boars, and, to our knowledge, no study is available from France. We aimed at assessing the HEV RNA prevalence in wild boar livers from south-eastern France.

**Methods:** Between September 2007 and January 2008, liver samples were collected from 285 wild boars hunted in the Bouches-du-Rhône (n = 278) and the Var departments (n = 7). Total viral RNA was extracted from 200μl of each wild boar liver sample following homogenisation in sterile phosphate-buffered saline, then clarification, using the MagNA Pure LC RNA Isolation Kit. HEV RNA detection and sequencing were performed using in house real-time PCR and
amplification/sequencing assays targeting the 5' ORF2 region of the HEV genome. Genotype/subtype was determined using phylogenetic analysis. Results: HEV RNA was detected using real-time PCR from liver of seven (2.5%) of the 285 wild boars. HEV sequences were obtained in five cases, and belong to genotype 3f. They showed 89–100% nucleotide identity with each other, and 80–98% identity with genotype 3 HEV sequences obtained from human hepatitis E cases diagnosed in the Microbiology laboratory of Marseilles public hospitals. Phylogenetic analysis showed that they clustered together with human and pig HEV sequences from France and Spain. Moreover, a strong phylogenetic link could be found between three wild boar HEV sequences from the present study and sequences obtained from a French patient and from swine manure in Spain. Conclusion: Our results indicate that wild boars, together with domestic pigs, might represent an HEV reservoir in southern France, and suggest that wild boars should be a potential source of HEV transmission for humans in this geographical area.

**P2046** Investigation on the presence of WU and KI polyomaviruses in central nervous system samples

L. Squarzon, M. Pacenti, V. Melitello, M. Treciun, P.G. Scotton, L. Burzon, G. Polia (Padua, Treviso, IT)

Objectives: WU and KI polyomaviruses have been recently discovered in respiratory secretions from patients with acute respiratory tract infections, where they are generally detected in 4% and 2% of cases, respectively, often as coinfection with other respiratory viruses. However, their association with human diseases remains still unclear. Aim of this study was to investigate whether WUV and KIV are detectable in central nervous system (CNS) samples and, in case of positive results, to understand their possible association with neurological diseases.

Methods: The presence of WUV, KIV, and JCV DNA was retrospectively investigated by real-time PCR in cerebrospinal fluid (CSF) samples from 60 consecutive patients (26 females and 34 males; median age 44 years; range 0–88) with neurological signs and symptoms suggestive of acute or chronic viral encephalitis and in 25 paraffin-embedded CNS samples from 16 HIV-positive asymptomatic subjects (median age 31 years; range 25–40) who died of acute opiate intoxication. CSF samples were selected among those submitted to routine PCR screening for the presence of neurotropic viruses in the period from January 2008 to April 2008, but without clear identification of causative viral agents.

Results: CSF samples from 2 patients (F 5 yr and M 63 yr), both with B-cell lymphoma, were positive for JCV DNA besides EBV DNA. All CSF samples were negative for KIV DNA, whereas a CSF sample from a 42-yr-old male tested positive for WUV DNA. The patient was HIV-positive and had clinical and radiological signs of progressive multifocal leukoencephalopathy (PML). All autopic CNS samples were negative for KIV and WUV DNA but positive for other common neurotropic viruses.

Conclusion: While extending our study to further CSF samples and to brain biopsies, these preliminary results raise the possibility that WUV may be associated with PML.

**P2047** A surveillance study of paroviruses from animals in Hong Kong discovered two novel paroviruses closely related to human parovirus 4

J.S.Y. Chan*, S. Lau, P. Woo, H. Tse, C. Fu, H.W. Toot, K. Li, B.J. Zheng, K.Y. Yuen (Hong Kong, HK)

Background: Paroviruses are a family of viral pathogens with a wide animal tropism. However, within the vertebrate specific sub-family Parovirinae, only a few members were known to be associated with human disease, these are parovirus B19, adeno-associated viruses, and more recently, the human bocaviruses and human parovirus 4 (PARV4). PARV4 is an interesting novel human parovirus since it is found to have less than 30% amino acid similarity to other paroviruses forming a distinct branch in the phylogeny of the Parovirinae sub-family. Since zoonosis is a common theme in many emerging infectious diseases, we sought to identify PARV4-like viruses in animal samples. Identifying possible animal origins of PARV4 is important to the understanding of its epidemiology and evolution, both of which are still poorly understood for this novel human parovirus.

Methods: Animal specimens were collected directly from slaughter houses or pig farms and from wet markets in Hong Kong. A variety of specimens were collected from the animals where possible, these include lymph nodes, faecal, nasopharyngeal, and serum samples from 303 pigs. Additionally, 30 liver and spleen samples, from pigs and cattle respectively, were also collected for the study. DNA extraction followed by PCR was performed to identify PARV4-like viruses. Of the samples positive for PARV4-like viruses, ten samples (7 from pigs and 3 from cattle) were chosen for partial genome sequencing.

Results: Of the porcine samples, a positive rate of 10% to 71% was found among the different specimens with lymph nodes and faecal samples having the highest and lowest positives respectively. Of the bovine spleen samples, 13% were found to be positive for PARV4 like viruses. Partial genome sequencing of the porcine and bovine strains showed a 62% and 63% nucleotide identity to PARV4 respectively. Analysis of the genome organisation and phylogenetic analysis of the genome sequences suggest the presence of two distinct novel paroviruses that are closely related to PARV4 where the three formed a distinct cluster among other paroviruses.

Conclusion: Two novel animal paroviruses closely related to PARV4 were found. From the genomic organisation and phylogenetic analysis, we propose that these two novel paroviruses, together with PARV4, to form a separate genus.

**P2048** Crimean-Congo haemorrhagic fever among healthcare workers in Turkey


Objectives: Crimean-Congo Haemorrhagic Virus (CCHFV) infection causes a fatal haemorrhagic syndrome, which is a leading threat to public health in endemic countries. Since 2002, 3128 CCHF cases were reported to the Ministry of Health of Turkey. Health care workers (HCW) are under occupational risk of CCHF infection.

Methods: Occupationally infected 7 HCWs with CCHF during the 2002–2009 epidemic in Turkey were investigated. All of these HCWs did not have exposure to the CCHFV via other routes such as tick bite. The transmission routes, clinical course, laboratory findings, and the management of the cases were described.

Results: Between 2002 and 2009, 272 CCHF cases were admitted to our department. Seven of these cases were HCWs, and they were exposed to virus during care of CCHF patients. Four of 7 HCWs were working in our hospital and the other 3 were infected in other healthcare settings located at the CCHF epidemic region of Turkey. The routes of infection were described as: exposure of infected blood to skin and mucosa, injury with needle stick contaminated with CCHFV, entubation of the infected patient, placement of nasal tamponade for prevention of bleeding of a patient. The lack of compliance to the standard precautions was considered as the main factor for the acquisition of the CCHFV infection. Ribavirin treatment was given to all the cases and one of them died.

Conclusion: Standard precautions and contact and droplet precautions are known to be sufficient for the protection from CCHFV infection during routine care of CCHF patients. However, these precautions should be strictly applied by all HCWs.

**P2049** Crimean-Congo haemorrhagic fever in Turkey


Objective: Crimean-Congo haemorrhagic fever (C-CHF) is severe viral disease affecting multiple organ systems. It is caused by infection with

Methods: The study was performed as retrospectively in adults patients diagnosed with C-CHF in Haydarpasa Numune Training and Research Hospital between 2006 and 2008.

Results: The mean age of patients (male 2, female 6) was 52.7 years. The mean incubation period of the disease (from the bite of an infected tick to onset of symptoms) was 5.6 days. The patients admitted to our hospital from the other provinces of eastern Turkey. 3 of 8 patients were from Giresun, and the other 2 were from Ordu, Kastamonu (1 patients), Gümüşhane (1 patients), and Sivas (1 patient). All these patients had high fever. Other clinical features were as follows: weakness (75%), myalgia (75%), headache (38%), petechial rash (38%), nausea (25%), loss of appetite (25%), vomiting (13%), abdominal pain (13%), gingival bleeding (13%), epistaxis (13%). The laboratory results showed leukopenia in all patients, thrombocytopenia in 7 of 8 patients. Serum aspartate aminotransferase (AST) (median 107 U/L, <37), alanine aminotransferase (ALT) (median 117 U/L, <42), were elevated in all patients. Lactate dehydrogenase (LDH) (median 636 U/L, >37) were elevated in six patients. Creatine phosphokinase (CPK) levels were elevated in 4 of 8 patients. Methods of diagnosis included antibody detection by enzyme-linked immunoasay (ELISA) in four patients. Serum sample of one patient was positive by RT-PCR. Three serum samples were positive for both ELISA and viral genome detection by RT-PCR. All patients were treated with oral ribavirin and supportive therapy. All the patients were cured.

Conclusion: C-CHF is characterised by haemorrhage, myalgia and fever, with case-fatality rate of up to 50%. The lowest case-fatality rate was reported from Turkey. There were no deaths among these patients. Tick bite have been the major transmission routes in this report.


S.A. Ahmeti* (Pristina, AL)

Crimean-Congo Haemorrhagic Fever (CCHF) is acute viral zoonosis that appears after tick bite in endemic areas during months April–July. Objectives: The aim of this study was to represent special clinical, epidemiological and laboratory characteristics of CCHF in our cases caused by native Hoti-Kosova virus.

Methods: There are studied 207 patients with Crimean-Congo haemorrhagic fever serologically confirmed (from totally 564 cases with clinical manifestations) which are treated at the Infectious Diseases Clinic of the University Clinical Center of Kosova during period 1989–2008. Diagnosis of the disease is set based on serological, clinical and laboratory data and is confirmed by serological-viral tests (ELISA, RT-PCR).

Results: With viral examinations, in cooperation with Microbiological Institute of Ljubljana, is isolated a new virus, causer of CCHF in Kosova and is nominated Hoti-Kosova virus, which is different in phylogenesis from other regional types of CCHF viruses. Infection is caused after tick bite. Human transmission is found in 21.2% cases inside family, and in 4.2% of cases between health personnel. Infection is more frequent in males (57.9%) than in females. Diseases has shown severe course with intensive haemorrhagic manifestations: petechie (69.3%), bloody eyeball (49.2%), epistaxis (62.4%), haematemesis (70.5%), melena (78.2%), haematritis (29.2%), metorrhagia (34.5%), liver disorders like hepatitis and mild renal disorders. Specific manifestations in our cases are: otorrhagia (2.03%), Horpus Febrilis (6.6%), haemoptoeritoneum (13.2%), pleuritis haemorrhagica (5.6%), pericarditis haemorrhagica (8.6%) and thrombocytosis during period of convalescence (1.5%). There has been high rate of Fatality (23.83%).

Conclusion: Republic of Kosova is endemic zone for CCHF. Disease is caused from native type of virus Hoti-Kosova, closely related to a CCHF virus strain Drosdov. Course of the disease has been severe with specific clinical manifestations and with high Fatality Rate.

Clinical findings and laboratory data in Crimean-Congo haemorrhagic fever as a re-emerging diseases

H. Salehi*, M. Rostami, F. Khorsash (Isfahan, IR)

Introduction: CCHF (Crimean Congo Haemorrhagic Fever) is a zoonotic disease which arises from animals such as sheep, goat and cow as sources and the main vector is tick (Hyalomma). This disease as a reemerging one appeared in Asia, middle east (especially in Iran) and since 2001 is going to become a major health problem in these area. Being aware of epidemiological and clinical features about it may be essential.

Precise description of clinical signs may help to pick up patients in prodromal phase especially in sporadic situation. We are going to analyses the gathered data of our 8 years cases, according to their answer sheets.

Material and Methods: We had 75 suspect cases during Nov 2001–Oct 2008 who referred to our hospital (Alzahra) as referral centre. For each patient 2 separate blood samples were sent to Institute pastur of IRAN. Each sample was examined for specific IgG, IgM & RT PCR. Of 75 cases simultaneously an answer sheet filled for every patient includes: Data of Epidemiological, clinical manifestation and course of disease. Study only includes confirmed cases.

Results: 30 cases (75%) had specific IgM antibody and positive PCR and they were confirmed cases.
The most important signs and symptoms in order of frequency in confirmed cases were: Fever in 30 cases (100%), petechia & purpura in 26 cases (83%), myalgia in 25 cases (83%), Malaise in 25 cases (83%), Haematuria in 20 cases (62%), Echymoses in 10 cases (32%), Icterus in 5 cases (12%), dry cough in 3 cases (8%), Abdominal pain in 2 cases (5%). Nearly all patients had one of the below Epidemiological factor: Close contact with slaughtered animals, close contact with animal wastage or Tick Bite.

All the contacts happen during last 7–10 days.

Lab data in order of frequency in confirmed cases were: Thrombocytopenia 30 cases (100%), AST & ALT elevation in 23 cases (75%), CPK rising in 18 cases (60%), leukopenia in 14 cases (45%) Anaemia in 11 cases (35%), PT & PTT disorder in 6 cases (20%), Proteinuria in 6 cases (20%), Leukocytosis in 5 cases (15%), BUN & creatinin rise in 3 cases (10%), chest X Ray abnormalities were seen in 2 cases (5%). 23 patients (75%) cured with Ribavirin & supportive care, but 7 (22%) died because of massive Haemorrhage.

Conclusion: CCHF as a viral haemorrhagic fever is scheduled as a re-emerging and urgent disease which is prevalent in Asia and specially middle east. Health care workers and hospitals have to be aware and stand by for managing patients with this disease.

**P2053** Preliminary study on immunological reactivity in people occupationally exposed to tick-transmitted pathogens

**J. Chmielewska-Budson**, V. Zajac, E. Cisak, J. Zwoinski (Lublin, PL)

**Objectives:** Our past research on prevalence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Babesia microti* in ticks in the Lublin region proved that the people attending forest are exposed to these pathogens. The aim of the study was to evaluate the infection rate with these pathogens in people inhabiting this region and occupationally exposed.

**Methods:** Sera collected from 49 forestry workers, 39 farmers and 32 blood donors were examined with ELISA IgM and IgG for antibodies to *B. burgdorferi*, *A. phagocytophilum*, *Bartonella* spp. and *B. microti* were detected with IgG immunofluorescence assay kits.

**Results:** Antibodies to *B. burgdorferi* were found frequently in forestry workers and in farmers 21/49 (42.9%) and 15/39 (38.5%). *Bartonella* was also frequent in forestry workers and less in farmers 20/49 (40.8%) and 9/39 (23.1%), respectively. Rarely, but also more frequently in forestry workers were found antibodies to *Babesia* and *Anaplasm* equally in 6/49 (12.2%) whereas in farmers only in 1/39 (2.6%) and 2/39 (5.1%), respectively. In the control group (blood donors) antibodies to *Bartonella* were the most frequent antibody found 12/32 (37.5%) with antibodies to *Borrelia* found in 4/32 (12.5%) and in 3 cases *Anaplasma* was detected (9.4%). In total, antibodies to one or more examined factors were found in 35 forestry workers (74.4%), 19 farmers (48.7%) and in 18 persons belonging to the control group 56.3%. Analysing the frequency of co-seroprevalence of examined factors with chi-squared test showed statistically significant differences between group of forestry workers and the control group (16/49 vs. 1/32, p<0.01) and forestry workers and farmers (16/49 vs. 4/32, p<0.05).

**Conclusion:** The results show frequent infections with tick-transmitted pathogens in occupationally exposed people.

Acknowledgement: The study was supported by the Polish Ministry of Science and Higher Education, Grant No. N N404 029135.

**P2054** Prevalence of Pneumocystis jirovecii colonisation in the general population of southern Spain: a preliminary study


**Objectives:** The epidemiology of Pneumocystis in human populations is largely unknown. A previous study conducted in our country has shown that *P. jirovecii* DNA can be detected in the respiratory tract of immunocompetent healthy adults. The objective of this preliminary study was to know the prevalence of Pneumocystis colonisation in the general population of Southern Spain.

**Methods:** This prospective community-based study included non-selected 132 children and adults from a rural area of Seville (El Coronil), evaluated at the local outpatients clinic for routine checkup or minor symptoms. Each participant underwent a clinical-epidemiologic examination. Identification of *P. jirovecii* colonisation was done analysing gargled oropharyngeal wash samples by nested-PCR assay that amplifies the mitochondrial large-subunit RNA.

**Results:** The mean age of persons was 52.9±21.6 years (range: 3–88) and 95 (72%) were male. Pneumocystis colonisation defined by detecting *P. jirovecii* DNA in a person without signs or symptoms of pneumonia was found in 10.6% of cases (14/132). All carriers were adults and had normal total lymphocyte and leukocyte cell counts. Twelve of them were asymptomatic at the time of their enrolment in the study. One of the carriers had been diagnosed with neoplasm and had taken steroids before the study and the remaining 13 had not undergoing lung disease or immunosuppression. Mean age of Pneumocystis carriers was higher than mean age of noncarriers (66.2±17.3 vs. 51.2±21.5, p = 0.01). No differences were detected due to sex and smoking habit between carriers and noncarriers.

**Conclusion:** This study confirms that *P. jirovecii* DNA can be frequently detected in the respiratory tract of immunocompetent persons, which agrees with the hypothesis that the general population could be a reservoir and source of this infection. Immunocompetent carriers in community ecosystems may present a public health issue that merits further research.

Supported by CIBER de Epidemiologia y Salud P ´ublica and ERA-NET Pneumocystis-PathoGenoMics.

**P2055** Giardia and Cryptosporidium in Finland – who gets reported?

**R. Rimmahen-Finne**, M. Kuusi (Helsinki, FI)

**Objectives:** The first water and food-borne outbreaks of giardiasis and cryptosporidiosis were detected in Finland, 2007 and 2008. In order to find out who gets reported with *Giardia* and *Cryptosporidium* infection during peace-time, we describe the characteristics of giardiasis and cryptosporidiosis cases notified to the Finnish Infectious Disease Registry (FIDR).

**Materials:** Notification of laboratory confirmed Giardia and Cryptosporidium infections to FIDR is mandatory in Finland since 1995. The characteristics of persons with notifications between 1 January 1995 and 31 December 2006 were analyzed according to age, gender, place of residence, seasonality and travel history. Data on country of birth was available for notifications between 1 January 2004 and 31 December 2006. The definition ‘Finn’ included persons who were born in Finland.

**Results:** During 1995–2006, 3390 *Giardia* and 130 *Cryptosporidium* infections were notified. The average yearly incidence was 54 giardiasis and 2 cryptosporidiosis cases/million inhabitants. No clear seasonality was observed. Both genders were equally present; the median age was 22 years for giardiasis and 26 years for cryptosporidiosis cases. In Helsinki metropolitan area, the incidence of giardiasis was 3-fold and that of cryptosporidiosis 12-fold compared to other parts of Finland. Data on travel history was limited, but most cases, in which data on travel history was available, reported travelling abroad. Majority of the giardiasis and all cryptosporidiosis cases were Finns. Among Finns, the incidence of giardiasis was highest in the age group of 20–29 years, whereas in non-Finns the infections were most common in <5 year olds.

**Conclusions:** Giardiasis and cryptosporidiosis are mainly reported in young adults living in the metropolitan area. These persons possibly travel more, but this could not be verified due to the limited data on travel history. The high incidence of giardiasis in young non-Finns probably reflects the frequency of immigration examinations. Compared to the reported incidences of giardiasis and cryptosporidiosis in the neighbouring countries, the base line incidences in Finland appear low. Traditionally, *Giardia* and *Cryptosporidium* have been considered as...
Detection of ESBLs, AmpCs and MBLs

**P2057** Rapid detection of extended-spectrum β-lactamase-producing Enterobacteriaceae: a randomised, investigator-blinded evaluation of culture-based approaches

S. Malhotra-Kama*, J. Cortiñas Abrahantes, C. Lammens, G. Molenbergh, M. Aerts, H. Goossens on behalf of the MOSAR WP2 Study Group

**Background:** Rapid and accurate detection of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-En) is crucial for effective infection control. We assessed 4 chromogenic media-ChromID (bioMérieux), CHROMagar (CHROMagar Microbiology), Amber (AES Chemunex), and a yet to be introduced formulation, Chromogenic-ESBL (Oxoid) – and 1 selective medium – EbSA (Alpha-Omega) – for their ability to correctly identify ESBL-En using well-characterised isolates and spiked stool samples.

**Methods:** Eighty-four samples consisting of 16 ESBL-En (E. coli, K. pneumoniae, Enterobacter spp., P. mirabilis, P. aeruginosa harbouring CTX-M, SHV, TEM or PER), and 5 non-ESBL-En (E. coli, K. pneumoniae, Enterobacter spp., P. mirabilis) at concentrations of 10^4 CFU/ml and 10^5 CFU/ml, respectively, and each of the 21 isolates spiked into stools at 3 concentrations (10^4, 10^5, 10^6 CFU/ml) were randomised and spiral plated on the 5 media. Media were read by 5 blinded investigators for characteristic colonies after 24 and 48 hrs incubation. One putative ESBL-En colony from the selective medium and 1 colony of each colour/type from the chromogenic media for each plated sample was confirmed for species identification on biochemical tests and for presence of ESBL by double-disc synergy test. Mean sensitivity (SEN) and specificity (SPEC), and confidence intervals (CI) were estimated for each medium by logistic regression model based on reader response for both incubation times, and both at the aggregated (any ESBL-En detected) and penalised level (correct species-colony colour correlation), using the penalised likelihood approach.

**Results:** Chromogenic-ESBL showed almost equal to 100% mean SEN and SPEC at both 24 and 48 hrs with the aggregated reader response and narrow CIs indicating a high precision of these parameter estimates (Table). Although-Chromogenic-ESBL also showed the highest SEN and a high SPEC with the penalised reader response for both incubation times, these values were lower than the aggregated response primarily due to misclassifications of *E. aerogenes* (TEM) and *P. aeruginosa* (PER) based on colony colour. Mean SENs for the other 4 media increased on average by 6.5% from 24 to 48 hrs. EbSA and ChromID showed almost equal to 100% mean SPECs at both incubation times, and the latter also with both reader responses.

**Conclusions:** Chromogenic-ESBL showed the best performance overall irrespective of sample concentration, reader or incubation time.

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**Table 1: Testing of different infectious diseases from immigrants**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Tests</th>
<th>% of respondents who had tested for pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhlococcus</td>
<td>Choriase antigen</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Tuberculosis antigen</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Hepatitis C</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>HIV</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Syphilis</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Treponema Pallidum Haemagglutination Assay</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Treponema Pallidum Antigluta</td>
<td>22</td>
</tr>
</tbody>
</table>

**Table 2: Sensitivities and specificities of media for ESBL-En detection**

<table>
<thead>
<tr>
<th>Media for ESBL-En detection</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>EbSA (Alpha-omega, BE)</td>
<td>79.0%</td>
<td>99.6%</td>
</tr>
<tr>
<td>CHROMID (bioMérieux, FR)</td>
<td>94.1%</td>
<td>94.7%</td>
</tr>
<tr>
<td>CHROMagar (CHROMagar Microbiology, FR)</td>
<td>75.0%</td>
<td>93.1%</td>
</tr>
<tr>
<td>Amber (AES Chemunex, FR)</td>
<td>54.4%</td>
<td>65.5%</td>
</tr>
<tr>
<td>Chromogenic-ESBL (Oxoid, UK)</td>
<td>94.9%</td>
<td>95.6%</td>
</tr>
<tr>
<td>Reader response aggregated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocci</td>
<td>71.9%</td>
<td>82.1%</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>48.7%</td>
<td>56.8%</td>
</tr>
<tr>
<td>Reader responses penalized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocci</td>
<td>71.9%</td>
<td>82.1%</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>48.7%</td>
<td>56.8%</td>
</tr>
</tbody>
</table>

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**P2056** Infectious diseases of immigrants – present screening systems in Finland

A. Pulakka*, H. Nohynek, P. Ashorn (Tampere, Helsinki, FI)

**Objectives:** The aim of this master’s thesis study is to acquire detailed information on the current practices, applicability and acceptability of infectious disease (ID) screening of immigrants in different health service facilities in Finland. Suggestions on how to improve the prevailing screening practices is also asked.

**Methods:** The study is a cross-sectional survey utilising mixed-mode data collection method. Data is primarily collected with an electronic semi-structured questionnaire but a paper-and-pencil version of the questionnaire is also available. Participants are health care professionals who work with immigrants in different settings: primary health care facilities, services for refugees, reception centres for asylum seekers, student health care facilities and occupational health clinics. Health care providers from 20 different municipalities are included, representing municipalities that received 64% of all immigrants and 76% of all refugees who came to Finland during 2007. Data collection will be done from October 2008 until the end of January 2009.

**Preliminary results:** Preliminary results are derived from answers of 73 respondents of whom 8 are medical doctors and 65 other health professionals: public health nurses, nurses and midwives. Over half of respondents consider ID screening very useful both for the immigrants and the society. ID testing is done to all immigrant groups and in all health care facilities. Most commonly screened IDs are hepatitis B and HIV (Table 1). Testing has identified cases of hepatitis B, tuberculosis, HV and syphilis. 52% of the respondents are satisfied with existing instructions to conduct screening although 69% of the respondents want to have new instructions and 86% state that more education is needed.

**Conclusion:** In Finland, ID screening is done to different immigrant groups and in different health care setting. Health care professionals consider screening to be useful but new instructions and education is requested.

**Table: Means sensitivities and specificities of media for ESBL-En detection**

<table>
<thead>
<tr>
<th>Media for ESBL-En detection</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>EbSA (Alpha-omega, BE)</td>
<td>79.0%</td>
<td>99.6%</td>
</tr>
<tr>
<td>CHROMID (bioMérieux, FR)</td>
<td>94.1%</td>
<td>94.7%</td>
</tr>
<tr>
<td>CHROMagar (CHROMagar Microbiology, FR)</td>
<td>75.0%</td>
<td>93.1%</td>
</tr>
<tr>
<td>Amber (AES Chemunex, FR)</td>
<td>54.4%</td>
<td>65.5%</td>
</tr>
<tr>
<td>Chromogenic-ESBL (Oxoid, UK)</td>
<td>94.9%</td>
<td>95.6%</td>
</tr>
</tbody>
</table>

**Reader response aggregated**

| Cocci | 75.0% | 82.1% |
| P. aeruginosa | 48.7% | 56.8% | 48.7% | 56.8% |

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**P2058** A laboratory evaluation of chromogenic screening media for the detection of extended-spectrum β-lactamase producing bacteria

A.J. Brown*, S. O’Brien, S. Cocks, J. Bracca, S. Dimmer (Basingstoke, UK)

**Objectives:** Since the 1980s, the increasing incidence of plasmid-encoded extended-spectrum β-lactamases (ESBLs) has been of major concern. The prevalence of ESBL-producing bacteria across Europe is not well understood and is currently being studied by an EU project; Mastering hOSpital Antimicrobial Resistance (MOSAR). Treatment options for infections caused by bacteria possessing such plasmids are limited due to their resistance to β-lactams, monobactams and cephalosporins. In vivo resistance to aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole has also been widely reported, leaving carbapenems as the currently preferred therapeutic option. Routine screening for ESBL-producing Enterobacteriaceae is becoming more widely adopted. Traditional culture-based screening is labour
Comparison of the chromID™ Evaluation of ESBL producing enterobacteriaceae: strains and the cost-saving due to reduced work-up of identification of non-ESBL producing organisms. The main advantages of chromID™ ESBL over CTAZ were the direct identification of E. coli and the cost-saving due to reduced work-up of identification of non-Enterobacteriaceae strains.

**Methods:**
A total of 80 pure culture isolates, comprising ESBLs (n = 23), non-ESBL chromosomal AmpC (n = 13) and other non-ESBL producing organisms (n = 44) were prepared as suspensions equivalent to 0.5 McFarland turbidity standard. Each ESBL isolate was serially diluted to provide an inoculum of 10 to 100 cfu from a 50 microlitre volume, which was spread over the surface of each medium. The negative organisms were inoculated directly from the 0.5 McFarland suspension using a 10 microlitre loop and the diminishing sweep technique. All plates were incubated aerobically at 37°C for 24 hours.

**Results:**
Both media obtained sensitivity and specificity of >90% in this study. ChromID ESBL correctly identified 21 of 23 ESBLs and 52 of 57 non-ESBL. Brilliance ESBL agar correctly identified 22 of 23 ESBLs, 49 of 53 non-ESBLs. Each medium also enabled differentiation of E. coli and coliforms from other Gram-negative bacteria due to enzymic cleavage of specific chromogenic substrates.

**Conclusion:**
Chromogenic screening media can provide earlier presumptive identification than traditional culture-based methods. Both media examined in this study achieved high sensitivity and specificity within 24 hours.

**[P2059]** Comparison of the chromID™ ESBL medium and MacConkey agar supplemented with ceftazidime (5 mg/l) for the detection of extended-spectrum β-lactamase producing Enterobacteriaceae from rectal swabs in hospitalised patients

C. Nonhoff*, H. Rodriguez-Villalobos, M.J. Struelens (Brussels, BE)

**Objective:**
 Rapid identification of patients colonised with extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae is useful for the early detection and control of nosocomial outbreaks. The aim of this study was to evaluate the clinical diagnostic performance of the selective chromogenic agar medium chromID™ ESBL (bioMérieux, Marcy-l’Etoile, France), compared with our in-house medium CTAZ (MacConkey agar + ceftazidime 5 mg/l) for the detection of ESBL-producing Enterobacteriaceae from rectal swabs in ICU hospitalised patients.

**Methods:**
Hospitalised patients (n=299) were screened for ESBL carriage by sampling rectal swabs (n=436) from 5/11 to 17/12/2007. After homogenisation by vortexing for 15 s, 100 μl of swab was inoculated onto chromID™ ESBL and CTAZ. Plates were incubated at 35°C for 18 and 48 h. Identification and susceptibility testing were performed by using the Vitek 2 system. The presence of ESBL was confirmed by combined double disks according to CLSI guidelines. Genotypic characterisation was determined by PCR assays targeting βlaTEM, βlaSHV and blCTX-M genes. Isolates harbouring βlaTEM and βlaSHV were further analyzed by sequencing to identify the ESBL.

**Results:**
Of 95 Enterobacteriaceae strains isolated from 48 patients (16.1%), 69 ESBL-positive strains were found in 32 patients. These included Enterobacter cloacae (n=38), Enterobacter cloacae (n=20), Klebsiella pneumoniae (n=8) and other (n=3). CTX-M derived enzymes (n=53) were frequently encountered in E. coli and E. cloacae. TEM (n=10) and SHV (n=6) were found in E. coli, K. pneumoniae and Enterobacter spp. The sensitivities/specificities were 89.9/96.7% and 84.1/94.3% for chromID™ ESBL and CTAZ, respectively. CTX-M positive isolates were significantly more frequently recovered on chromID™ ESBL while TEM and SHV-producing strains were more frequently isolated from CTAZ (p<0.001). chromID™ ESBL was more selective against non-ESBL-producing Enterobacteriaceae isolates whereas CTAZ was more selective against other Gram-negative bacilli.

**Conclusions:** chromID™ ESBL and CTAZ media demonstrated equivalent performance in terms of sensitivity and specificity for the detection of ESBL-producing Enterobacteriaceae. The main advantages of chromID™ ESBL over CTAZ were the direct identification of E. coli and the cost-saving due to reduced work-up of identification of non-Enterobacteriaceae strains.
Detection of ESBLs, AmpCs and MBLs

**Results:** Among the *E. coli* strains we found 503 (8%) strains producing ESBLs. Comparing the MIC distributions for cefotaxime of ESBL and non ESBL strains (fig.) the differences were striking: No strains with MICs >8 mg/L in the non ESBL group, few with MICs between 4 and 16 mg/L in both groups (mutation of chromosomal β-lactamase in non ESBL group). High number of strains above 16 in the ESBL group. Strains with an MIC of 2 were about 15 times higher in the ESBL group, indicating a high number of ESBLs with low MICs. Although EUCAST and CLSI breakpoints for amoxicillin/clavulanate differ for 16 mg/L in both groups (mutation of chromosomal ESBLs. Comparing the MIC distributions for cefotaxime of ESBL and non-ESBL strains (9.1%) were ESBL producers. The MIC distributions showed a range of different β-lactams because of the different specificity of the β-lactamases.

**Conclusion:** To detect ESBL producing strains of *E. coli* and *Klebsiella* with the first susceptibility test laboratories should test very low concentrations of 3rd generation cephalosporins (as low as the epidemiological cut off point) since even the EUCAST breakpoints may fail to detect ESBL producing strains with low MICs. Another help is the use of a cut off point since even the EUCAST breakpoints may fail to detect ESBL producing strains with low MICs. Another help is the use of a cut off point since even the EUCAST breakpoints may fail to detect ESBL producing strains with low MICs.

**Methods:** All consecutive single-patient isolates from 2003 through 2007 that fulfilled criteria of the Clinical Laboratory Standards Institute (CLSI) and/or European Committee on Antimicrobial Susceptibility Testing (EUCAST) for an ESBL phenotype were included. Cefepoxide, ceftriaxone, ceftazidime and aztreonam as well as cepheope were used for screening. Molecular characterisation of all isolates was performed by PCR and by DNA sequencing for the most common ESBL types including CTX-M, TEM, and SHV as well as the non-ESBL types such as SHV hyperproducer (SHV HP) and OXA-1. Epidemiological data were prospectively collected in standardised case report forms.

**Results:** A total of 110 strains met the phenotypic study criteria for ESBL. Only 70% (77/110) of phenotypic ESBL strains were confirmed by molecular analysis, whereas 68.8% (53/77) were expressing a CTX-M gene. Among the phenotypically 30% false-positive ESBL strains, 45.4% (15/33) were SHV-1 HP and 30.3% (10/33) OXA-1. In SHV-HP, only ceftazidime was positive in the ESBL screening and confirmation tests, and these strains were resistant to β-lactamase-inhibitor combinations (BLCI). OXA-1 strains had an elevated cepheope MIC, a positive ESBL confirmation test with cepheope, but were negative for other screening compounds and also resistant to BLCI. *E. coli* strains were more likely to represent true ESBL than other species (OR 5, 95% CI 1.25–20). False-positive ESBL were more prevalent in patients with fatal comorbidities (p = 0.014) and cultures sites other than the urine (p = 0.016).

**Conclusions:** Use of tests in addition to the recommendations of CLSI may improve the sensitivity for detection of ESBL, but go along with a lower positive predictive value in a low-endemicity setting. The most important group of false-positive ESBL were SVH-1 HP and OXA-1. The most common false positive ESBL results could be detected by applying additional phenotypic criteria.

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**Detection of extended-spectrum β-lactamase among Enterobacteriaceae using automated microbiology systems**


**Objectives:** The prevalence of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae has increased considerably in recent years. Detection of the ESBL-phenotype is necessary to allow an accurate interpretation of susceptibility results and to guide antimicrobial therapy. Therefore confirmatory tests and corresponding software algorithms have been added or improved in commercially available semiautomated microbiology identification and susceptibility testing systems.

**Methods:** To evaluate their ability to detect ESBL production in Enterobacteriaceae, two of these systems, i.e. the VITEK2 System (bioMérieux, Marcy l’Etoile, France), and the MicroScan WalkAway-96 System (Siemens Healthcare Diagnostics, West Sacramento, CA), using the current routine testing panels which include ESBL detection and confirmation tests, were compared. A total of 147 isolates of *Escherichia coli* (n = 61), *Klebsiella pneumoniae* (29), *K. oxytoca* (16), *Enterobacter cloacae* (17), *E. aerogenes* (6), *Citrobacter freundii* (5), *Citrobacter freundii* marcescens (1), and *Proteus* spp. (12) were distributed blindly to two participating laboratories. All of these had been previously characterised by disk approximation method, the CLSI double-disk synergy test, the Etest ESBL and a biochemical and molecular characterisation of β-lactamases at an independent laboratory. Isolates included 95 ESBL producers and 52 non-ESBL produces such as hyperproducers of chromosomal AmpC, Koxy, or SHV enzymes, and wildtype strains.

**Results:** The sensitivity, specificity, negative (NPV) and positive (PPV) predictive values were determined (see Table).

**Conclusion:** These results indicate that both systems are highly reliable for the detection of ESBLs in *E. coli*. The test performance was also good for *K. pneumoniae* isolates but not reliable for ESBL detection in *K. oxytoca* and the *Citrobacter*–*Enterobacter*–*Serratia* isolates.

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**Detection of AmpC enzymes and the prevalence of plasmid-mediated AmpC resistance genes**

C. Nic, Phogartaigh*, G. Vanstone, I. Balakrishnan (London, UK)

**Background:** β-lactamase resistance due to chromosomal AmpC enzyme production has been described in several members of the Enterobacteriaceae family and is a growing problem in hospital settings. Of greater concern, increasing numbers of plasmid-mediated AmpC (pmAmpC) genes have been discovered in nosocomial isolates of *E. coli* and *Klebsiella pneumoniae*. Detection of AmpC production is critical in order to optimise antibiotic therapy and clinical outcomes. There are no recommended CLSI guidelines for detection of this resistance...
mechanism. The detection of pmAmpC genes has further implications for infection control.

Objectives: To investigate local prevalence of AmpC producing Enterobacteriaceae, compare phenotypic methods of detection, and determine the extent of pmAmpC production.

Method: 226 consecutive Enterobacteriaceae isolates were included. Identification and sensitivity testing was performed using Phoenix method. Phenotypic testing for extended spectrum β-lactamases (ESBL) and AmpC production (including inducibility) was performed on all isolates using, respectively, 4th generation cephalosporin +/− clavulanate agar disc-diffusion synergy, and indicator cephalosporins cefoxitin, with cefazidime to detect inducible AmpC (current laboratory practice). In addition, a novel synergy-based method using ceftaxime +/− boric acid as an AmpC inhibitor 1 (an increase in zone size of ≥4 mm or more defining a positive result) was carried out, with the addition of cefoxitin to detect inducibility. All isolates were then tested using multiplex PCR for pmAmpC genes 2.

Results: The majority of isolates were urinary; 185 E. coli, 18 Klebsiella sp., 7 Citrobacter sp., 7 Proteus sp., 6 Enterobacter sp., 2 Serratia sp., and 1 Providencia sp. By the standard ESBL/AmpC detection method, there were 11 derepressed AmpC only (4.9%), 6 AmpC and ESBL (2.7%), 5 inducible AmpC (2.2%), and 14 ESBL only (6.7%). The 17 AmpC isolates consisted of 12 E. coli, and 1 each of K. pneumoniae, E. cloacae, E. aerogenes, C. freundii, and P. mirabilis. A comparison with the boronic acid synergy-based method is shown in the table.

Table: Comparison of standard ESBL/AmpC detection with a boronic acid synergy-based AmpC detection method.

<table>
<thead>
<tr>
<th>Standard detection method</th>
<th>Boronic acid synergy-based method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AmpC+</td>
</tr>
<tr>
<td>AmpC+</td>
<td>7</td>
</tr>
<tr>
<td>ESBL and AmpC+</td>
<td>4</td>
</tr>
<tr>
<td>Inducible AmpC</td>
<td>0</td>
</tr>
<tr>
<td>ESBL only</td>
<td>7</td>
</tr>
<tr>
<td>Neg</td>
<td>0</td>
</tr>
</tbody>
</table>

Isolates with discrepant results have been sent to the Antibiotic Resistance Monitoring Reference Laboratory for further characterisation of resistance genes. Multiplex PCR for pmAmpC yielded 2 positives: C. freundii, with only inducible AmpC on phenotypic testing, and a negative boronic acid synergy test, carried a CIT plasmid; and an E. coli identified as derepressed AmpC by both phenotypic methods carried a DHA plasmid. This suggests a presence of pmAmpC genes in 11.8% of AmpC producing organisms, though this will vary with genus.

Conclusions: This provides useful data on laboratory detection of AmpC, although we cannot make firm conclusions until reference laboratory data is available. Surveillance of pmAmpC resistance is necessary to monitor this emerging problem as infection control practices may need to be reviewed.

P2066 Routine susceptibility testing methods may fail to detect plasmid-mediated AmpC enzymes in Enterobacteriaceae

T.Y. Tan*, L.S.Y. Ng, J. He, D.M.M. Chen (Singapore, SG)

Objectives: Plasmid-mediated AmpC enzymes in E. coli, Klebsiella spp. and Proteus spp. confer resistance to third-generation cephalosporins. This study determined the accuracy of cephalosporin susceptibility testing against Enterobacteriaceae carrying plasmid-mediated ampC genes using three methods: disk diffusion, Vitek 2 and microbroth dilution.

Methods: 72 isolates of E. coli (n=53), Klebsiella spp. (n=15), and P. mirabilis (n=4) were tested by a multiplex PCR assay detecting 6 families of plasmid-borne ampC. Susceptibility testing was performed for cefotaxime, ceftiraxone and ceftazidime by disc diffusion following CLSI guidelines and microbroth dilution (Microscan, Siemens Diagnostics, USA). Automated susceptibility testing to ceftazidime and ceftiraxone was performed using the N019 card, on the Vitek 2 Compact (bioMérieux, France) running system software version 62.011. All susceptibility breakpoints were interpreted according to 2008 CLSI guidelines with the exception of the Vitek 2, which used the latest available installed 2005 CLSI guidelines, with expert interpretation and modification of susceptibility test results via the proprietary Automated Expert System (AES, version 1.9).

Results: CTX-M-like genes (n=59) were predominantly found in E. coli and P. mirabilis, and DHA-like genes in Klebsiella spp. (n=13). 20 (28%) isolates were susceptible to all three cephalosporins by microbroth dilution, as compared to only 13 (18%) by disk diffusion. Phenotypic resistance was highest in ceftazidime, with only 21 (29%) and 17 (24%) susceptible by microbroth dilution and disc diffusion respectively. Cephalosporin susceptibility interpretation was different for the Vitek system, as susceptibilities to ceftazidime and ceftiraxone are inferred using the AES. When the AES was disabled, 30 (42%) and 46 (64%) of isolates tested by Vitek 2 were susceptible to ceftazidime and ceftiraxone, respectively. When interpretation by the Vitek AES was subsequently applied to the testing results, 12 (17%) isolates were reported as susceptible to both cephalosporins.

Conclusions: Conventional susceptibility testing using existing CLSI breakpoints to third-generation cephalosporins fails to accurately detect some Enterobacteriaceae carrying plasmid-mediated ampC genes. The development of accurate screening methods will improve detection and reporting of this emerging resistance mechanism.
Cefditoren versus ceftazidime in inducer-substrate combinations for the evaluation of AmpC production in a disk approximation test


**Objective:** To evaluate cefditoren (CDN) in inducer-substrate combinations to screen for AmpC induction.

**Methods:** 100 clinical isolates (25 Pseudomonas aeruginosa, 25 Enterobacter cloacae, 14 Morganella morgani, 13 Serratia marcescens, 12 Citrobacter freundii, 7 Providencia rettgeri, and 4 Enterobacter aerogenes) were tested by the Kirby-Bauer disc approximation method using CDN and ceftazidime (CAZ) discs as substrates, and CDN and imipenem discs as inducers. Photographs of the incubated plates were taken using visualisation Gel Doc and inhibition zones were measured using the ImageJ program. A positive induction was considered when the inhibition zone of the substrate disc was reduced by ≥2 mm.

**Results:** None of the strains showed induction of AmpC with CDN-CAZ as inducer-substrate combination. Imipenem-CDN as inducer-substrate combination was not useful for evaluating strains of *P. aeruginosa* since no inhibition zones surrounding the cefditoren disc were found. Number (%) strains showing reduction when using CDN and CAZ as substrate, and mean±SD reduction in the inhibitory zone for valuable strains (those showing inhibitory zone) among the enteric bacteria tested, are shown in the Table.

**Conclusion:** CDN showed no induction capability, and when used as substrate (with imipenem as inducer) it offered detection rates of AmpC inducible enterobacteria higher than the imipenem-CAZ combination, mainly for *Enterobacter* spp. and *Serratia* spp., with higher diameter reductions for indol-positive proteae.

### Table: Comparison of ampC production in P. aeruginosa isolates

<table>
<thead>
<tr>
<th>Strain</th>
<th>Reduction (mm)</th>
<th>Reduction (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. freundii</em></td>
<td>6.0 (0.7)</td>
<td>4.17 (1.41)</td>
</tr>
<tr>
<td><em>M. morgani</em></td>
<td>10 (70.0)</td>
<td>3.9 (6.9)</td>
</tr>
<tr>
<td><em>P. aerogenes</em></td>
<td>5 (4.3)</td>
<td>3.8 (6.1)</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td>11 (100.0)</td>
<td>3.6 (10.5)</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>23 (15.2)</td>
<td>3.9 (6.4)</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>3 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>62 (47.2)</td>
<td>3.75 (2.0)</td>
</tr>
</tbody>
</table>

Results: Among the 86 isolates of *P. aeruginosa* resistant to carbapenems, 3 were positive by EIM assay, 5 by E-test, 38 by EDS and 68 by DD. The table describes the estimated sensitivity, specificity, positive predictive value and negative predictive value.

**Conclusion:** The EDTA disk synergy test (EDS) and synergy test with double disc of imipenem (DD) are rapid and sensitive methods for MBL production screening in *P. aeruginosa* but lacks specificity. The EDTA-imipenem microbiological assay (EIM) displayed excellent specificity but showed the lowest sensitivity among the analyzed procedures. E-test had a performance comparable to the gold standard.

### Table: Detection of ESBLs, AmpCs and MBLs

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of specimens</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDS assay</td>
<td>Positive</td>
<td>5</td>
<td>33</td>
<td>100</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD assay</td>
<td>Positive</td>
<td>5</td>
<td>63</td>
<td>100</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EIM assay</td>
<td>Positive</td>
<td>3</td>
<td>0</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-test</td>
<td>Positive</td>
<td>5</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>81</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Introduction:** Resistance to carbapenems due to MBL production in Enterobacteriaceae is increasingly recognized. Different levels of resistance to carbapenems have been observed in these isolates and reproducible susceptibility testing results within the same or among different methods is not always obtained. We studied the carbapenems susceptibility profiles of VIM-1 producing *K. pneumoniae* isolates belonging to the same clone which was involved in an epidemic in our hospital (2005–2008) affecting 18 patients.

**Methods:** Eighteen VIM-1 producing *K. pneumoniae* isolates belonging to an epidemic clone (Xbal-PFGE) were studied. Carbapenems MICs obtained by microdilution (CLSI), WIDER semiautomatic system, and Etest were compared. Errors in the clinical interpretive categories were determined considering microdilution as reference. Other mechanisms contributing to carbapenems resistance, such as porin expression, were also studied. Heteroresistance was determined by population analysis profile (PAP) in 4 selected strains displaying different imipenem MICs.

**Results:** Imipenem MICs were in the range of 8–128 mg/L by microdilution, ≥1–8 mg/L by WIDER and 0.75–32 mg/L by Etest. High error rates were observed with WIDER and Etest when compared with microdilution. The category agreement was 28% for WIDER and 11% for Etest with 28% of very major errors in both cases. Low reproducibility of MICs was observed with Etest, even when the same inoculum was used (up to 4-fold dilutions of difference). Heteroresistance for imipenem was initially suspected due to the presence of colonies in the inhibition zone of Etest strips. This was confirmed by PAP results obtained in all selected strains with the exception of the porin deficient *K. pneumoniae* isolate that homogeneously expressed carbapenems resistance.

**Conclusions:** Low reproducibility of MIC results to carbapenems obtained by different susceptibility testing methods could be due to the presence of resistant subpopulations in carbapenemase producing enterobacterial isolates. Variable expression of resistance mechanisms affecting carbapenems might also contribute to this effect. Carbapenems MICs are not good markers of MBL production and reliable phenotypic methods are needed to confirm the presence of this mechanism.
Rising carbapenem resistance in *Pseudomonas* and Enterobacteriaceae at a large district hospital in northwest England – how relevant is antimicrobial disc susceptibility testing?

A. Galeri, E. Trautt, B. Lunt*, R. Palmer (Blackpool, UK)

**Objectives:** Since Jan 2008, use of fluoroquinolones [FQ] and 3rd generation cephalosporins [ESC] has been restricted as part of hospital strategy to reduce rates of MRSA, ESBL and *Clostridium difficile* infections [CDI]. An increase in infections with carbapenem resistant [CR] *pseudomonas* and Enterobacteriaceae has been observed. Limitations of disc susceptibility testing [DST] has been reported. This study was undertaken to compare DST with MIC determined by Etests [AB bioMerieux] in CR Enterobacteriaceae and *Pseudomonas*; MBL production and Doripenem MIC was tested by E-tests.

**Results:** Results of 28 of total 100 isolates [to be presented] are included here: Of the 14 CR *Pseudomonas* isolates, disc-testing correlated with MIC results in 58 of 70 total tests (83% of all tests). 13 isolates were resistant to meropenem (93%) and only 1 was identified as an MBL producer using MBL strips (7%). 12 of the 13 meropenem resistant isolates were sensitive to ceftazidime (92%). Of the 14 enterobacteriaceae isolates, disc-testing correlated with MIC interpretation in 59 of 70 total tests (84% of tests). 3 isolates intermediate on Ertapenem disc testing were sensitive based on MIC (50% of ertapenem tests), 3 isolates ertapenem intermediate on disc testing were resistant based on MIC (21% of Ertapenem tests). Doripenem Etest results ranged 0.023–0.38 for Enterobacteriaceae 2,500 isolates. The majority of *Pseudomonas* sp in this study were not MBL-producers and this suggests a different mechanism of resistance. This has implications for therapy with other agents such as ceftazidime, which would otherwise not have been deemed effective. Ertapenem disc-interpretation differed from MIC in the majority of isolates. This would suggest the MIC interpretation of Ertapenem is more reliable than disc susceptibility testing. Isolates appeared 100% susceptible to Doripenem despite lack of defining BSAC cutoff points. Doripenem MICs [to be presented] are lower than Meropenem and Imipenem.

**Community-acquired respiratory tract infections**

**P2071** A prospective serological and virological prevalence study of influenza infection and immunisation at a metropolitan centre, northern Italy. A basis to re-evaluate immunoprophylaxis strategies for influenza in the childhood

R. Manfredi*, E. Ubaldini, F. Bernardi, A. Moroni, M. Donati, F. Chiudo (Bologna, IT)

**Introduction:** Anti-Influenza vaccination is presently considered a relevant public health issue also in children aged less than 2 years [MMWR 2006; 55:1062].

**Patients and Methods:** Starting from November 2005, a prospective serological-virological monitoring of all children hospitalised due to inflammatory/ infectious diseases after Emergency Room access, is ongoing.

**Results:** During the first 18 months of observation, 265 patients aged 6 months to 17 years were evaluated; out of 1,774 overall hospitalised children. Only 22 children (aged 2–11 years) (8.3%) had a positive IgA serology for Influenza A (4 of them had a concurrent positive IgA serology for Influenza B). IgG antibodies assays (prior immunity) were positive in only 9 c (Influenza A) (3.4%), while all direct viral search on respiratory secretions tested negative in all cases, save one. From October 2006 to December 2006, we recognized 14 out of 22 cases of IgA seropositivity, while in the remaining 15 months only 0–2 cases per months were found. Of 22 children with positive IgA serology, 18 (77.3%) attended a community or a school.

**Conclusions:** Although actively searched through systematic serologic and virologic assays, Influenza infection seems a proportionally rare event among children. Further studies which compare different laboratory techniques are needed to confirm our preliminary data, which however show an unexpected, reduced prevalence of Influenza infection in a broad paediatric population hospitalised for inflammatory/infectious diseases. A reappraisal of the phenomenon, sustained by strong microbiological date (and not limited to clinical evidences, as currently performed in common clinical practice) could lead to a revision of current vaccination strategies.

**P2072** The effect of psychological and psychosocial factors on the outcome of or susceptibility to acute respiratory tract infections

M. Falagas, C. Karamanidou, G. Karlis, A. Kastoris*, P. Rafailidis (Athens, GR)

**Objective:** The association between psychological and psychosocial factors and the onset and progression of acute respiratory tract infection, is unclear. We aimed to perform a systematic review of the available literature in order to assess the possible effect that, psychological or psychosocial variables may have on the susceptibility and/or the outcome of acute respiratory tract infections.

**Methods:** We performed searches on PubMed, Scopus, and PsychInfo. Articles eligible for inclusion contained quantitative data regarding the association between psychological or psychosocial variables and the onset or progression of acute respiratory tract infections.

**Results:** We identified 46 studies, published between 1986 and 2008, examining the role of psychological or psychosocial variables and the onset or progression of acute respiratory tract infection. Ten studies included the same study population although they assessed different psychological or psychosocial variables. Of the 46 studies included in our review, 41 showed at least one statistically significant association between psychological, psychosocial, or behavioural variables and susceptibility to acute respiratory tract infection. Eighteen out of 46 studies revealed at least one statistically significant association between psychological, psychosocial, or behavioural variables and outcome of acute respiratory tract infection. Variables associated with a higher risk of infection but also a less favourable outcome were depression, anxiety, negative affect, higher levels of perceived stress, more negative life events, anxious or angry mood states, and the personality trait neuroticism. Positive personality traits on the other hand, decreased the risk of infection, showing a more favourable effect on the outcome of infection.

**Conclusion:** Most of the studies included show a significant relationship between psychological, psychosocial, or behavioural variables and onset or progression of acute respiratory tract illness. The psychological or psychosocial variables measured are not consistently measured across the included studies, and different methodological approaches were used to examine the association between psychological or psychosocial factors and acute respiratory tract illness. Thus, more research is necessary in order to contribute to a better understanding of the association between psychological or psychosocial factors and susceptibility and outcome of acute respiratory infections

**P2073** Distribution of Legionella in hot water systems of hospitals and health resorts in Poland

A. Sikora, M. Wójtowicz*, A. Magrysz, M. Koziol-Montewka (Lublin, PL)

**Objective:** Poland introduced the legal regulation of controlling water systems for the presence of *Legionella* bacteria only in 2008. This project
was undertaken to evaluate occurrence of Legionella spp. in hot water systems in Polish hospitals and health resorts. **Methods:** Forty eight hot water samples (1000 ml each) were collected from 3 hospitals and 2 health resorts. All samples were collected from hot tap outlets, selected intermediate sites – depending on the length of the water system and distal sites. The samples were analyzed using the International Standard Method, accepted in Poland as a standard based on filtration procedure and culture of bacteria on selective media. The filtration method was performed with subsequent volumes of water samples: 10, 100 and 500 ml for accurate determination of Legionella number. In cases of samples with high contaminant flora, the smaller water volumes were filtered: 1 ml, 10 ml and 100 ml. The filters were then placed on the selective GVPC agar plate and incubated at 37°C in a humid atmosphere for 7 days. Suspected Legionella colonies were subcultured onto BCYE agar for verification. The species serogroups were determined by a commercially available latex agglutination test kit. **Results:** L. pneumophila SGs 2–14 were predominant and were detected in 72.9% of all analyzed hot water samples. L. pneumophila SG 1 was not detected in any of the tested samples. The numbers of legionellae detected exceeded 102 cfu/100 ml in 65.3% of the samples. Legionella contamination of water distribution systems was higher in examined hospitals for 2 hospitals (56.7%) and in health resorts (56.3%) as compared to 3 hospitals (38.8%). **Conclusions:** A large public buildings with complex water distribution systems represent ideal locations for Legionnaires’ disease transmission. To reduce the likelihood of Legionnaires’ disease transmission in health care facilities, a strategy focusing on proper maintenance of water systems and investigation of situations in which transmission has been shown to occur is strictly recommended. The higher consumption of hot water needed for treated bathing waters as well as specific quality of natural waters might be the reason of lesser Legionella colonisation of water system in health resorts comparing to hospitals.

**P2074** The effectiveness of different methods eradicking Legionella spp. from water distribution systems in selected hospitals of Lublin region

M. Wójtowicz*, A. Sikora, A. Magryś, M. Kozioł-Montewka (Lublin, PL)

**Objective:** Poland introduced the legal regulation of controlling water systems for the presence of Legionella bacteria only in 2008. The results presented have unique values indicating the level of Legionella contamination in hot water distribution systems in cases of lack of inspection. The aim of the work was to study occurrence of Legionella pneumophila in water distribution systems of three hospitals in Lublin region as well as eradication efficacy by using different decontamination methods. **Methods:** Forty hot water samples (1000 ml each) were collected from examined hospitals. The samples were analyzed using the International Standard Method (PN-ISO11731–2), accepted in Poland as a standard based on filtration procedure and culture of bacteria on selective media. **Results:** All water samples tested for the presence of Legionella spp. gave positive results. The numbers of legionellae detected exceeded 10^6–10^9 colony forming units per 100 milliliters in 70% of the samples. All of the positive samples contained L. pneumophila SGs 2–14, as detected by latex agglutination method. The major interventions included performing thermal eradication by permanent increase in the hot water temperature (≥55°C) and temporal increase in the temperature up to 70°C for 2 hours. All shower heads and sink taps in the hospitals were scalded out. Hot water temperature was monitored in selected points of water sampling. As a result, thermal disinfection was not satisfactory. Elimination of “dead ends” and application of sodium hypochlorite allowed to reduce the number of Legionella in hospitals’ water systems. However total elimination of bacteria by single action was not possible. **Conclusions:** It was found that: Legionella pneumophila SGs 2–14 colonise hospital hot water distribution systems in the amounts exceeding acceptable norms, thermal disinfection is not satisfactory, it is required to perform chemical disinfection by using chemical preparation of chlorine (chlorine dioxide, sodium hypochlorite), there is a need to eliminate “dead ends” in water systems.
findings, disease rates, serotype distribution and antimicrobial suscepti-
bility.

Results: 1,386 cases of laboratory-confirmed IPD were reported. Bacteraemic pneumonia, the most common clinical finding, was diagnosed in 63% of IPD cases. Annualised incidence rates of IPD in Alaska and northern Canada were 18 and 27 cases per 100,000 persons, respectively (rates among indigenous persons: 47 in Alaska and 35 cases per 100,000 persons in northern Canada). Rates in children <2 years of age were 109 and 156 cases per 100,000 persons in Alaska and northern Canada, respectively (rates in indigenous children: 251 in Alaska; 170 in northern Canada). IPD rates in children <2 due to PCV7 serotypes declined by >80% after routine vaccination with PCV7 (Alaska: 130 to 11 cases per 100,000 persons, p < 0.001; northern Canada: 129 to 37 cases per 100,000 persons, p < 0.001). Rates of disease with non-PCV7 serotypes in children <2 increased in Alaska (25 to 76 cases per 100,000 persons, p < 0.001), and in northern Canada (41 to 74 cases per 100,000 persons, p=0.17). Rates of IPD with penicillin-nonsusceptible isolates decreased from 63 to 27 cases per 100,000 children in Alaska (p = 0.001), and from 10 to 8 cases per 100,000 persons (p < 1.00) in northern Canada.

Conclusions: The high IPD rates among Arctic Indigenous people have declined in Alaska and northern Canada following PCV7 introduction. An increase in non-vaccine type disease of the magnitude seen in Alaska was not observed in northern Canada. Continued surveillance is needed to determine the impact of PCV7 and future higher valency conjugate vaccines when they come into use.

**P2078** Genetic polymorphisms of innate immunity and susceptibility to pneumococcal infection


Objective: To investigate whether diverse genetic variants of innate immunity (MBL, TLR2, TLR4, and FcgRIIa) that cause hyporespon-
siveness to S. pneumoniae might be associated with a higher risk of invasive pneumococcal disease in adults.

Methods: All adult patients with community-acquired Streptococcus pneumoniae disease admitted to the hospital from January 2005 to November 2007 were enrolled in this prospective study. Patients with congenital immunodeficiencies, HIV infections and severe neutropenia (<500 cells/mm³) were excluded from this protocol. Controls were patients of the same hospitalisation area and similar age range with negative blood culture and without previous history of pneumonia or meningitis. After obtaining written consent, we performed genotype analysis with 5 ml of peripheral blood and extract the DNA with (High Pure PCR Template Preparation Kit, Roche). By PCR-RFLP were detected MBL allelic variant B (ID:rs1800450), C (rs1800451) and TLR2 Arg753Gln (rs5743708). The MBL D variant (rs5030737), and Arg131His FcgRIIa, were performed by PCR. TLR4 Asp299Gly (rs4986790) was determined by sequencing the specific region. For statistical analysis, categorical variables were analysed using the chi-
square test and Fisher's test when appropriate. Continuous variables were compared using the Mann-Whitney U. Significant differences were defined as p < 0.05.

Results: One hundred and eighteen patients were included; of them, 75 patients (63.5%) were bacteraemic. The source of the pneumococcal disease were pneumonia 98 (83.1%), meningitis 18 (15.3%) and abdominal focus 2 (1.7%). Of them, 74 patients (62.7%) were admitted to the ICU, and 40 patients (34.2%) developed septic shock. The mortality rate was 18.8% (22 patients). The median age of the patients was 60 years±23 IQR and the median age of the controls (n = 52) was 55 years±28 IQR (p = 0.213). TLR4 Asp299Gly polymorphism was present in 25.4% of patients with pneumococcal disease and in 0% of control subjects (p = 0.001). Frequency of the other variant alleles was similar in infected patients and controls.

Conclusions: Among the assessed genetic variants of innate immunity, only TLR4 Asp299Gly polymorphism is associated with a higher risk of invasive pneumococcal infection in adults.

**P2079** Bacteraemic pneumococcal pneumonia in adults: infecting serotypes and mortality

V. Pascual Granollers*, E. Calbo, M. Xercavins, M. Riera, M. Rodríguez-Carballeira, J. Garau (Terrassa, ES)

Introduction: A secular trend in the epidemiology of infecting serotypes in bacteraemic pneumococcal pneumonia (BPP) has been reported. Heptavalent pneumococcal conjugate vaccine (HPCV) was licensed in Spain in the second semester of 2001. The aim of our study was to describe the evolution of serotypes distribution and its potential impact on mortality in adults with BPP over a 15 years period in our institution.

Methods: From 1993–2007, all adult patients with BPP identified through the records of the Microbiology Laboratory were included. Data recorded were: demographics, co morbidities, serotypes, antimicrobial susceptibility and in hospital mortality. Serotypes were analyzed individually and classified as vaccine serotypes (VS), vaccine related serotypes (VRS) and non-vaccine related serotypes (NVR) in relation to HPCV. Serotypes were also classified as of high invasive potential (HIP: 1, 5, 7) and low invasive potential (LIP: 3, 6A, 6B, 8, 19F and 23) and analysed accordingly. Three periods were distinguished: 1993–1997 (P1), 1998–2002 (P2) and 2003–2007 (P3). Patients were divided in three age groups 18–65 (A1), 66–79 (A2), and >80 years (A3).
Results: A total of 419 patients with BPP were included, 96 (23%) in P1, 136 (32%) in P2 and 187 (45%) in P3; 60%, 51% and 70% were males in P1, P2 and P3, respectively (p < 0.05). Median age was 71 y in P1, 71 y P2 and 63 y in P3 (p < 0.05). One or more co morbidities were present in 50% of patients in P1, 52% in P2, and 56% in P3 (NS). In P1, 10% of BPP were due to VS and 90% to NVR; in P2, 23%, 4% and 73% were due to VS, VRS, and NVR, respectively, and in P3, 13%, 4% and 78% were due to VS, VRS and NVR, respectively (p < 0.05). In P2, S1 was present in 22% in A1, 6.1% in A2 and 3% in A3 (p < 0.05) and HIP represented 29%, 10%, and 5% in A1, A2 and A3, respectively (p < 0.05). In P3, 17% in A1, 8% in A2 and 3% in A3 were due to S1 (p < 0.05) and HIP were responsible for 30%, 17% and 5% in A1, A2 and A3, respectively (p < 0.05). Mortality rates were 15%, 13% and 1% in P1, P2 and P3, respectively (NS).

Conclusion: NVR serotypes have been the predominant infecting serotypes causing adult BPP in our area throughout all the studied periods. Among all studied serotypes, only S1 and HIP were more frequently isolated in young patients in the two last periods. Despite of the changing epidemiology, in-hospital mortality of BPP has remained unchanged during the last 15 years.
more prolonged hospitalisation [7 (2–35) vs 6 (1–24) days; p < 0.05]. Seven out of the 80 diabetics (9%) died due to pneumonia, while all non diabetics survived (p < 0.01). In DM patients low serum albumin level upon presentation was associated with unfavourable outcome (p < 0.05).

**Conclusion:** Elderly diabetics with CAP require prolonged hospitalisation. Furthermore, in diabetics, despite the initial lower CURB65 score, mortality is higher, while low serum albumin upon presentation is predictor of unfavourable outcome.

**P2083** Bacterial respiratory tract infections as the sequelae of tuberculosis

A. Martynova*, A. Prushinskyi, J. Skurichina, E. Sokurova, K. Smirnov (Vladivostok, RU)

Despite of the results in treatment of tuberculosis, bacterial infections are the most often complications in the patients with pulmonary tuberculosis. We reported the causative microorganisms in children with remission of clinically confirmed tuberculosis.

**Aim:** Was to characterise the aetiology of bacterial respiratory tract infections in children with tuberculosis in anamnesis and also to compare the isolates gained from children with tuberculosis with isolates gained from healthy carriers.

**Methods:** The isolates of *H. influenzae* (n = 32), *S. pneumoniae* (n = 25), etc (n = 23) were isolated from lab material of 80 patients by the quantitative sputum culture method (greater than or equal to 10⁷/ml) in Far Eastern Pediatrics National Tuberculosis Center (control group (11 strains of *H. influenzae* and 17 strains of *S. pneumoniae*) was isolated from 40 healthy carrier children). Antimicrobial resistance was checked by disk-diffusion method and MIC.

**Results:** *H. influenzae* was the most frequent microorganism causing the bacterial infections in children with clinically confirmed tuberculosis. Antimicrobial resistance was a major problem only in children hospitalised with tuberculosis (rifampicin 21.8%, penicillin 40%, erythromycin 84.37%, co-trimoxazole 59.37%). The antimicrobial resistance pattern in healthy children was characterised as having less resistance to penicillin (1 strain), and there were no resistant strains to rifampicine. *S. pneumoniae* isolated in children with tuberculosis was resistant to rifampicin in 2 cases from 25 strains (8%), and in 36% to macrolides. The antimicrobial resistance pattern in healthy carriers had also no rifamicine resistant strains.

**Conclusions:** Presumed bacterial respiratory infections sequelae of tuberculosis should be diagnosed according standard criteria before starting antibiotic therapy, and treatment modified depending on culture results. Also it could help to prevent then with rational methods of vaccination.