Posters

Molecular typing – Part 1

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

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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 (iSEQ™). 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)

[1] Lefmann, M. et al. (2004). J Clin Microbiol 42(1): 339–46.

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

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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 37ºC 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

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Objectives: There is a geographic variation in *Helicobacter pylori* genotypes. cagA and cagE, oipA 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 oipA 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 ethnics including 7 Kurdish, 5 Lurs, 3 Afghani and 2 Arab patients. The predominant vacA signal region genotype was s1 among isolates from all ethnics. 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 oipA gene was present in 51.4% of Persian, 33.3% of Turks and 70.5 of others ethnics 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 oipA 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.

P512 The number of *Helicobacter pylori* CagA EPIYA C tyrosine phosphorylation motifs is associated with histological features of chronic gastritis

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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 scored according to the Sydney system. One biopsy specimen from the antrum was used for DNA isolation and *H. pylori* genotyping. The presence of cagA was determined by PCR and reverse hybridisation. The number and type of EPIYA motifs were determined by PCR using primers flanking the EPIYA-coding regions.
Results: *H. pylori* cagA-positive strains were present in 55 of the 117 patients (47.0%). In cagA-positive cases, the number of EPIYA C motifs ranged from 0 to 3: 11 cases (20.0%) had 0, 33 cases (60.0%) had 1, 9 cases (16.4%) had 2, and 2 cases (3.6%) had 3 EPIYA C motifs. The presence of cagA and the number of cagA EPIYA C motifs were associated with more severe chronic inflammation in the corpus, and higher grade of polymorphonuclear activity and presence of epithelial damage in both corpus and antrum. The magnitude of risk for gastric atrophy and intestinal metaplasia increased with increasing number of EPIYA C motifs: the odds ratio for atrophy was 5.4 (95% CI, 1.6–18) in individuals infected with strains <2 EPIYA C motifs, and was 12.1 (95% CI, 2.5–57) in individuals infected with strains with $\geqslant$ 2 EPIYA C motifs.
Conclusions: *H. pylori* cagA-positive strains and strains with $\geqslant$ 2 CagA EPIYA C motifs are associated with more severe histopathological features of the gastric mucosa, gastric atrophy and intestinal metaplasia. The characterisation of the CagA EPIYA-containing region may be important in more clearly defining risk for gastric pre-malignant and malignant *H. pylori*-associated diseases.

P513 *Helicobacter pylori* vacA intermediate region i1 strains are associated with more severe histological features of chronic gastritis and increased gastric carcinoma risk in Portugal

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Background and Aims: *H. pylori* vacA gene is present in all strains and is polymorphic at the s and m regions. Functionally, s1/m1 strains cause more extensive vacuolation and in a wider range of cell lines than s1/m2 strains, and s2/m2 strains are non-vacuolating. Clinically, s1/m1 strains are associated with gastric atrophy, intestinal metaplasia and gastric carcinoma. Recently, a new polymorphic region within vacA designated intermediate (i) region, was described as a major determinant of vacA toxicity, and i1 strains were associated with gastric carcinoma. The aim of this study was to characterise the i-region in strains infecting Portuguese patients in order to explore the relationship between i-region genotypes and the histological features of gastritis, and the risk for gastric carcinoma.
Materials and Methods: 154 *H. pylori*-infected patients were studied, 106 with chronic gastritis and 48 with gastric carcinoma. Histological parameters were scored according to the Sydney system. DNA isolated from gastric specimens was used directly for PCR. Genotypes were determined by reverse hybridisation on a line probe assay for vacA s and m regions, and by type-specific PCR for vacA i region.
Results: vacA i region was successfully genotyped in 97.4% strains. 53 (35.3%) cases were multiple for vacA s, m, or i regions. In the 97 cases of single vacA genotypes, 42 (43.3%) were s1/m1, 45 (46.4%) were s2/m2, and 10 (10.3%) were s1/m2. In agreement with previous descriptions, s1/m1 strains were predominantly i1 (41/42) and only one s1/i2/m1 strain was found. Also, the great majority of s2/m2 strains were i2 (44/45) and a single s1/i2/m1 strain was found. s1/m2 strains were genotyped as i1 (9/10) or i2 (1/10).
In chronic gastritis patients, vacA i1 strains were significantly associated with higher degrees of corpus chronic inflammation and polymorphonuclear activity ($P = 0.0006$ and $P = 0.002$, respectively). vacA i1 strains were also associated with the presence of corpus and antral epithelial damage (both $P < 0.0001$), and with glandular atrophy and intestinal metaplasia ($P = 0.0016$ and $P = 0.0043$, 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.

P514 Evaluation of use of multiple locus variable number tandem repeat analysis for typing of *Pseudomonas aeruginosa*

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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 at loci 172, 211, 214, 217, 222, 207 and 209. The Liverpool and Midlands 1 strains, isolated from multiple patients with cystic fibrosis (CF), could be identified unambiguously by their characteristic VNTR profiles at the 7 loci. This was also the case for other CF associated strains. There was some variation in repeat numbers at two of the 7 loci among isolates of clone C from different patients, but, otherwise, with the odd exception only, repeat numbers were consistent among representatives of a single PFGE type. The method successfully distinguished all 17 serotype reference strains, none of the profiles of which matched one another, or any of the panel or routine isolates tested. Repeat numbers at the eighth locus (61) 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.

P515 Comparison of opr sequences for the characterisation of clinical isolates of *Pseudomonas aeruginosa*

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Objectives: Allelic variation has been described in the genes encoding the outer membrane proteins of *P. aeruginosa* (Pirnay et al. 2002). OprD, oprI, and oprL show potential for use in the epidemiological typing of strains. We determined the partial oprD, oprI and oprL 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 $\geqslant$ 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.

P516 Molecular epidemiology of *Pseudomonas aeruginosa* in intensive care units over a 10-year period (1998–2007)

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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 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

	1998	2000	2003	2004	2007
No. of ICU admissions	2367	2446	2400	2554	2675
No. of cases	142	74	85	83	87
No. of cases/1000 admissions	60	30.3	35.4	32.5	32.5
Cat. 1	23.7	7.0	0.4	1.6	2.6
Cat. 2	16.5	6.5	11.3	8.2	10.1
Cat. 3	15.6	13.1	16.7	16.8	17.9
Not typed	4.2	3.7	7.1	5.9	1.9

P517 Assessing clonal relatedness of *Pseudomonas aeruginosa* isolates: MLVA should be preferred over MLST and PFGE

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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 97.4–1.0), 98.0 (CI 95.9–1.0) 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. Wc between PFGE and MLVA is 0.97 both ways. Wc 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 aRI 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

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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 early 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 SpeI-PFGE and MLST (Curran et al. 2004, http://pubmlst.org/paeruginosa) using the housekeeping genes acsA, aroE, guaA, mutL, nuoD, ppsA 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: SpeI-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*

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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 controll, 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 mehods. 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 patinets 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 mehods. 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

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Objective: 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′-GCGATCCCCA-3′), and by BanI 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 specie *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

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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 SmaI 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 candidial 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 Mutilocus sequence typing of sequential *Candida albicans* isolates from patients with persistent or recurrent fungaemia

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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 in 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 two or more blood cultures positive for *C. albicans* on 2 or more separate days. Recurrent 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, well 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. Significantly, 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*

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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 (94ºC), 30 seconds (94ºC), 2 minutes (20ºC) and 1 minute (72ºC) followed by 30 cycles of 30 seconds (94ºC), 30 seconds (30ºC) and 1 minute (72ºC). 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

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Background: *Clostridium difficile* (CD) is an important cause of communitary and nosocomial diarrhoea. 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 (ImmunoCard, Meridian Bioscience). DNA was obtained from pure cultures using Chelex resin (Instagene matrix, BioRad). The tcdA gene (toxin A), tcdB gene (toxin B), and binary-toxin genes cdtA and cdtB were detected by PCR following methods previously described (Kato, 1991; Wolfhagen, 1994; and Stubbs, 2000, respectively). Bin + isolates were characterised by PCR-ribotyping (Bidet 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 18 bp in 10 isolates (2 different ribotypes), 36 bp in 5 isolates (2 different ribotypes), 39 bp in 69 isolates (2 different ribotypes), and 54 bp 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.

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.

P525 Co-existence of multiple MLVA sub-types of *Clostridium difficile* PCR-ribotype 027 strains within faecal specimens

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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 specimens 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.

P526 First nationwide estimates on incidence and case fatality of *Clostridium difficile*-associated infections

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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.

P527 A rapid and simple identification of *Clostridium difficile* PCR ribotype 027 by loop-mediated isothermal amplification method

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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 (Killgore 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 the 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.

P528 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

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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 staphylococcal SCCmec genes type was done (by multiplex PCR) were determined.

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 antimicrobial classes, 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 (OR, 5.2; CI, 4.83–5.94) but overall we have very high index of resistance for MRSA as our most sensitive antibiotic was Trimethoprim-Sulfamethaxozol with 42% susceptibility (Table).

Resistance profile of MRSA strains according to epidemiological classification of MRSA

Antimicrobial agent	Number (%)			
	HA-MRSA	CA-MRSA	Total	*P*-value
Ciprofloxacin	68 (72)	5 (33)	73 (67)	0.003
Cotrimoxazole	60 (63)	4 (26)	64 (58)	0.007
Clindamycin	73 (77)	8 (53)	81 (74)	0.045
Rifampin	63 (67)	5 (33)	68 (62)	0.012
Tetracycline	78 (83)	6 (40)	84 (77)	0.00
Erythromycin	73 (77)	9 (40)	79 (72)	0.002
Doxycycline	58 (90)	4 (57)	62 (87)	0.011

Conclusions: Our study, as we know, was first study in Iran about differentiation of different characteristic of CA-MRSA and HA-MRSA. We have very resistant MRSA in our hospitals and this is troublesome for care of patient's most important point in for control of emerging infections is supervision in antibiotic use by infectious control committees.
Also we found as other studies difference in origin of CA-MRSA and HA-MRSA.

P529 Phenotypic and molecular characterisation of methicillin-resistant *Staphylococcus aureus* isolated from clinical samples in northern Chile

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Objectives: Methicillin-resistant *Staphylococcus aureus* is a significant problem in healthcare settings around the world. The aim of this work was to study the phenotypic and molecular characteristics of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from clinical samples in the north of Chile.
Methods: A total of 76 MRSA strains from skin and tissues infections samples isolated in Health Centers of Antofagasta, Chile (2007), were included in this study. The *S. aureus* strains were identified by biochemical tests, API Staph and typed by Phene-Plates. The susceptibility to antimicrobial drugs was determined by a dilution technique agar, the presence of beta-lactamase by phenotypic and molecular methods, and detection of mec and luk genes by PCR. Also, the presence of plasmids was investigated by gel electrophoresis and genotypes by PFGE.
Results: More of 80% of the assayed MRSA strains exhibited high resistance to ampicillin, penicillin, eritromycin, claritromycin, amikacine and ciprofloxacin; moderate resistance to cotrimoxazole (18.5%) and tetracycline (4.2%); and 100% of susceptibility to rifampin and vancomycin. Around 90% of the strains were resistant to methicillin and the 79% presented beta-lactamase. The Ph-phene method identified 7 biochemical phenotypes and the antibiotic resistance patterns shown 5 different antibiotypes. More of 60% of the strains exhibited multiple resistance to 9 or more antimicrobials. Different genotypes were identified by PFGE and one genotype was dominant. A plasmid of 7 kb in 8 MRSA strains, also two of them presented a plasmid of 20 kb. The PCR analysis revealed the presence of mecA gen in 97.3%, mecR1 MS gen in 52.6%, mecR1 PB gen in 36.8%, and mecI in 55.2% of the MRSA strains, and also in 44.5% of the strains was detected the luk gene. However, the mecA gen was found in 6 methicillin susceptible strains. In two MRSA strains, it was not possible to detect the presence of the mecA gen.
Conclusions: MRSA strains are present in a high percentage in Health Centers of Antofagasta Chile, the strains exhibited multiple resistance to antibiotics and presence of plasmids. Also, the MRSA strains presented beta-lactamase, mec and luk genes in prevalent phenotypes and genotypes of MRSA strains. These findings can contribute to understand and control the MRSA strains circulating in the Health Centers of Antofagasta.

P530 Molecular characterisation of methicillin-resistant *Staphylococcus aureus* isolates in a tertiary hospital in Madrid (Spain)

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Objectives: The aim of this study was to determine which clones of methicillin-resistant *Staphylococcus aureus* (MRSA) are circulating in our hospital and to analyse the genetic relationships of these strains.
Methods: We studied a total of 52 MRSA strains isolated from clinical samples of 52 hospitalised adult patients (10 patients were admitted to the medical/surgical intensive care unit, ICU, and 4 to the burn ICU) during 2007 at the Department of Microbiology of Getafe University Hospital in Madrid. The sources of the isolates were nares (65%), oropharyngeal (15%), rectal (7%) and wound (13%) swabs. The 52 MRSA were characterised by phage typing using the 23 phages of the Basic International Set at 100 RTD and 1,000 RTD, pulsed-field gel electrophoresis (PFGE) following SmaI digestion of chromosomal DNA as described by Cuevas et al. (Clin Microbiol Infect 2007;13: 250–256) and SCCmec typing wich was performed using several multiplex PCR strategies [1–3].
Results: Of the 52 MRSA isolates, 33 (63%) belonged to phage group III and 5 (10%) belonged to phage group III and also were lysed by phage 81. The rest (14 strains) were non-typeable. Genotyping by PFGE showed that the majority of the strains belonged to the clones E-7 (16 isolates, 31%) and E-8 (14 isolates, 27%) with the next subtypes: 5 strains belonged to E-7a, 8 to E-7b, 3 to E-7c, 8 to E-8a, 5 to E-8b and 1 to E-8c. We observed two isolates belonging to E-11, one to E-13 and one to E-20. The remaining 18 MRSA belonged to sporadic clones but 3 of these strains presented the same PFGE pattern and were isolated from 3 different patients who were admitted to the burn ICU at the same time.
SCCmecIV accounted for 46 (88%) of the isolates (76% IVa, 22% IVc and 2% IVh). The remaining 6 strains (12%) carried SCCmecI and all were sporadic clones.
Conclusions: The majority of the strains showed the most common PFGE types present throughout Spain (E-7 and E-8). The genotypes E7, E8, E11 and E20 belong to the clonal group 5 (paediatric clon). We also found one strain with the PFGE profile E-13 (SCCmecIVh) belonging to the EMRSA-15 clone which is epidemic in the United Kingdom. It is important to note that we observed an outbreak in the burn ICU of a sporadic clone carried SCCmecIVa.

Reference(s)

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

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Objectives: *Staphylococcus aureus* is a major pathogen responsible for bacteraemia and sepsis with extremely high mortality. Methicillin-resistant *S. aureus* (MRSA) has emerged since 1960 s 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 mecA (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 database of 9 medical centres. 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-typabe 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 ($p = 0.10$) was noted. Forty-one MRSA isolates were typed with MLST and 3 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 SCCmecIII-ST239-agrI-DRUs14 and SCCmecIV-ST59-agrI-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

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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 profiles a total of 24 different types were identified within the CC8 isolates. Unlike the SIRU profiles where the same profiles were present in both Pakistani 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 ST8 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

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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) PCR, staphylococcal protein A (spa) typing, 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 t011 and t108. SCCmec typing results of 300 NT-MRSA isolates showed SCCmec type IV, type V, and unknown type(s). Surprisingly, all isolates with spa type t108 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* analysing the hypervariable mec-associated direct repeat unit

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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. 14:964–69). 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.

Methods: The establishment of a new Internet-accessible database freely available at http://www.dru-typing.org/search.php.
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

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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 (t024; 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 af one spa type replacing another but more likely the co-existence of spa type variants with a common origin.

Reference(s)

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

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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

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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 2006/7 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.

P538 *Staphylococcus aureus* genotypes in patients with cystic fibrosis

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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). Identification and susceptibility testing were performed using standard procedures. PFGE method with digestion with SmaI 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.

Molecular virology

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

P. van Deursen, A. Verhoeven, P. de Bie, L. Bertens, J. de Jong (Boxtel, 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 testing is proposed as alternative for plasma testing. The NucliSENS easyQ HIV-1 v2.0 (in development, bioMérieux) supports plasma and dried blood testing.
Objectives: The aim of this study is to establish the analytical sensitivity and the linear range of quantification for HIV-1 RNA detection in dried blood spot specimens using NucliSENS EasyQ HIV-1 v2.0.
Methods: EDTA whole blood samples obtained from HIV-1 RNA negative individuals were spiked with different amounts of HIV-1 (range 0–70,000,000 VQA copies/ml). Spots of 50 µl were made on paper (ProteinsaverTM 903® Card, Whatman) and dried 3–24 hours. Next, two spots (0.1 ml blood) were extracted with the NucliSENS EasyMAG and analyzed with NucliSENS EasyQ HIV-1 v2.0 (including three batches) to determine analytical sensitivity by applying Probit analyses, and accuracy of quantification as compared to 0.1 ml plasma specimen processed in parallel. In addition, robustness testing was performed with an HIV-1 input concentration three times above the established limit of detection using three NucliSENS EasyMAG instruments, and on each instrument 129 samples were processed.
Results: The analytical sensitivity (95% detection rate) was 71 and 26 VQA copies/extraction for dried blood spot and plasma samples, respectively. Excellent linear correlation ($y = 1.00x - 0.05$ with $R2 = 0.99$, range 70–7,000,000 VQA copies/extraction) was found between DBS and plasma measurements, for all three batches included. Robustness testing at an HIV-1 RNA input concentration of 218 VQA copies resulted in 100% detection, supporting the limit of detection as indicated above.
Conclusions: In this study, NucliSENS EasyQ HIV-1 v2.0 was successfully validated for DBS specimen types, resulting in an analytical sensitivity of 71 copies, and accurate quantification in the range 70–7,000,000 copies. Next, additional clinical studies are required to evaluate the new method for monitoring anti-viral therapy responses in HIV-1 infected individuals.

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

P. van Deursen, A. Verhoeven, P. de Bie, J. de Jong (Boxtel, 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 µl spots were made on paper (ProteinsaverTM 903® Card, Whatman) and dried 3–48 hours. Next samples were stored at different temperatures (–20ºC, 5ºC, RT, 37ºC, and 55ºC). Samples stored at 37ºC were subjected first to shipment simulation; 5 days 37ºC (of which 8 hours high humidity at 37ºC and 8 hours high humidity at 55ºC), 3 days 5ºC, 5 days –20ºC, 3 days 5ºC, and subsequently 5 days 37ºC (of which 8 hours high humidity at 37ºC and 8 hours high humidity at 55ºC). High humidity conditions were tested at 37ºC and 55ºC. After storage HIV-1 RNA levels were measured using two spots (0.1 ml blood) and NucliSENS EasyQ HIV-1 v2.0.
Results: For the high input samples no or limited reduction in HIV-1 RNA levels ($\leqslant 0.30$ log10) was observed for all conditions tested with one exception (6 weeks 37ºC at high humidity). For the low input sample (70 VQA copies/extraction) the detection rate was $\geqslant 80\%$ 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 37ºC (with and without including shipment simulation), 3 weeks high humidity at 37ºC, 1 week high humidity at 55ºC, 3 weeks 4ºC, 1 week –20ºC, 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 37ºC for 9 weeks. Instability was observed after 6 weeks 37ºC high humidity. The results support the use of DBS specimen for accurate viral load measurements after transport and storage when taken these limitations into account.

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

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Objectives: The Siemens VERSANT®[a] HIV-1 RNA 1.0 Assay (kPCR)[b] 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.

Methods: Assay performance characteristics were evaluated at three laboratory sites, with each site using two VERSANT HIV-1 RNA 1.0 Assay (kPCR) kit lots and two instrument systems. Plasma samples from 1,055 HIV-1 seronegative individuals were tested to assess assay specificity. A 13-member dilution panel prepared from high titer HIV-1 8E5/LAV viral stock was used to determine precision, linearity, accuracy, quantification range and analytical sensitivity: panel members had HIV-1 RNA concentrations ranging from 10 to >11,000,000 copies/mL. The method comparison between the VERSANT HIV-1 RNA 1.0 Assay (kPCR) and the Abbott RealTime HIV-1 assay was conducted using 189 HIV-1 RNA-positive plasma samples that included HIV-1 Group M subtypes A, B, C, D, F, G, H; circulating recombinant forms AE and AG; and Group O variants. All samples possessed HIV-1 viral loads within the common assay range of 75 to 10,000,000 copies/mL.
Results: The overall assay specificity was 99.7%. The limit of detection (LoD) was 37 copies/mL: the lower limit of quantitation (LLoQ) was 37 copies/mL and the upper limit of quantitation (ULoQ) was 11,000,000 copies/mL. On average the VERSANT HIV-1 RNA 1.0 Assay (kPCR) generated values that were 0.21 log copies/mL lower than the Abbott RealTime HIV-1 assay. The Deming regression slope (fitting VERSANT log quantitation versus Abbott log quantitation) was 1.07.
Conclusions: The VERSANT HIV-1 RNA 1.0 Assay (kPCR) using the VERSANT kPCR Molecular System is a reliable and accurate assay for the quantification of HIV-1 RNA in plasma from HIV-1 infected individuals.
[a]VERSANT is a registered trademark of Siemens and all other trademarks are the property of their respective owners.
[b]CE marked in Europe; not available in the US.

P542 Comparison of commonly used real-time PCR methods to detect and quantify HIV-1 proviral DNA in infected patients

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Objectives: Different PCR methods have been widely used to quantify cell associated 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 commercial commonly 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).
Results: 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.

P543 HIV and HBV mutations in co-infected patients treated with lamivudine

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Objective: Hepatitis B virus (HBV) mutations conveying resistance to antiviral drugs are a major problem in the treatment of chronic hepatitis B. Information available on the prevalence and distribution of distinct HBV variants in HIV positive patients is scarce. Our aim was to investigate the frequency of mutations and variables potentially associated with an increased risk of liver disease evolution in HIV/HBV co infected individuals that have fail antiretroviral treatment.
Methods: For HBV genotype was determined using commercial Line Probe Assay (Innogenetics) and detection of hepatitis B virus mutations conveying resistance to lamivudine (LAM) was performed with INNO-LiPA HBV DR v2 (Innogenetics NV, Ghent, Belgium).
For HIV we sequenced the HIV pol gene with Trugene genotyping kits (Bayer Diagnostics Inc.), and for HIV subtyping purposes, all the nucleotide sequences were submitted to the Stanford database.
Results: We evaluated 9 patients, aged 20±1 years, HIV-HBV co-infected that have been treated with antiretroviral therapy including LAM for an average of 3.8±3years. HIV and HBV were parenterally acquired during childhood. The patients had no clinical signs of hepatitis. Median CD4 count was 143.6cells/mmc (range 8–490); median HIV ARN was 380278.5 copies/ml plasma (range 38000–1020000). We found a positive correlation between HBV-DNA and HIV-RNA in plasma ($r = 0.6$, $p < 0.001$). All patients were infected with the HIV F1 subtype, while HBV genotype was A. Patients had no HDV co-infection.
Analysing resistance to LAM in HIV viral strain we noticed that 6 patients had a high degree of resistance. The most common mutation in the RT gene was M184V/I that causes high-level in vitro resistance to LAM, but also confers a diminished HIV replicative fitness, associated with type 2 TAMs: D67N, K70R, T215F, and K219Q/E.
In HBV viral strain the most frequent mutation were: 11-L180/A181 in 8 patients; 18 M204 in 7 patients; 26-N236 in 7 patients; 15M180/A181 in 4 patients; 20-V204 in 4 patients; 20-V204 in 3 patients; 12L181/T181 in 2 patients. 55.5% of the patients were HBeAg positive with significantly more frequent LAM resistance mutations.
Conclusions: Comparing the frequency of mutations conveying resistance to LAM in 2 different viruses reveals a greater variety in HBV than HIV viral strain in spite of the absence of clinical signs of hepatitis and virologic failure toward HIV treatment. The presence of HBeAg may be a determinant of LAM resistant mutants.

P544 Human papillomavirus prevalence and type distribution in women before the introduction of vaccination in Italy

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Objective: Knowledge about the prevalence of human papillomavirus (HPV) on a population level is important to assess the potential impact of HPV vaccines. Aim of this study was to determine the baseline prevalence and type-specific distribution of HPV in women from the Northeast of Italy prior to the introduction of HPV vaccination.
Methods: Population-based study of prevalent type-specific HPV infection in consecutive women undergoing HPV testing at our Institution for cervical cancer screening program or opportunistic screening in the 5 year period from January 2004 to December 2008. HPV DNA was detected in cervical samples by MY09/MY11 and GP5+/GP6+-based PCR and genotyped by sequencing.
Results: Cervical samples from 2,978 women were analyzed: 1,153 (38.7%) tested positive for HPV DNA, 200 of which could not be genotyped because of the presence of multiple infections or low HPV DNA load. In the remaining samples, sequencing allowed to

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. Development of enhanced VLP vaccines with larger type coverage should take into consideration HPV epidemiological data.

P545 Prevalence and type specific papillomavirus load in female anal infection

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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 samples were 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 HPVs 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.

P546 Inter-laboratory reproducibility and precision of the Roche prototype Cobas® 4800 human papillomavirus test

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Background: The prototype cobas® 4800 is a highly automated system that performs sample preparation, real-time HR-HPV amplification and simultaneous detection of 12 HR-HPV genotypes in a single pool, with separate detection of HPV16, HPV18, as well as the human beta globin gene, all in single tube. Reproducible run to run and system to system performance is demonstrated using a multi-level PreservCyt panel with 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 39 target site, SiHa cells (HPV genotype 16), HeLa cells (HPV genotype 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.
Conclusion: The prototype cobas® 4800 HPV test system shows good between-lab reproducibility and within-run precision for both HPV16 and HPV18.

P547 Epstein-Barr virus 30 bp deletion variant of LMP-1 gene in paediatric liver transplant recipients and its association with clinical course

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Objectives: Paediatric liver transplant (LTx) patients (pts) are at particular risk for developing EBV related post-transplant lymphoproliferative disorders (PTLD) following administration of immunosuppressive therapy. LMP-1 is the main oncogene of Epstein-Barr virus. Wild type (wt) and 30 bp deletion variants of LMP-1 gene (30 del LMP-1) have been shown to differ in their molecular and biological properties (the latter being considered as more tumorigenic and less immunogenic). The aim of the study was to evaluate the relationship between these two LMP-1 variants and EBV DNA loads, response to reduction of immunosuppression (RIS), and PTLD development in paediatric LTx pts.
Methods: 67 pts after LTx were included in this study. Median age at LTx was 1.25 y (0.2–14.3 ys). The study involved 6 pts with histologically confirmed PTLD. Primary immunosuppression was tacrolimus (FK-506) plus mycophenolate mofetil (MMF) in 24 pts, FK-506 plus prednisone in 30 pts, 13 pts followed others schemes of IS according to established protocol for paediatric LTx. LMP-1 variants were detected in blood of 65 EBV DNA positive pts by PCR using primers flanking the site of characteristic 30 bp deletion. 40 pts have had EBV DNA load monitored using real-time quantitative PCR method. The results were reported as EBV DNA copies/ug PBMC DNA. High viral load pts (HVL pt; EBV DNA >2000 copies) received RIS with antiviral medications.
Results: The wt LMP-1 was detected in 52/65 (80%) pts, including 4 PTLD pts, whilst 30 del LMP-1 variant was detected in blood of 11/65 (17%) pts, including 2 PTLD pts. Both wt and 30-del LMP-1 were detected in blood of 2 (3%) pts. 13/40 pts had low viral load (median 675 copies). 27/40 pts received RIS plus antivirals due to high EBV DNA load (median = 40 421 copies). Statistical analysis showed no significant difference between LMP-1 variants and EBV DNA load

(Mann-Whitney test, p = 0.56) and no correlation between LMP-1 variant and PTLD development (Chi square, p = 0.62). Interestingly, significantly slower decrease in EBV DNA load in response to RIS was found in pts with 30 del LMP-1 variant (Mann-Whitney test, p = 0.016; median 15.05 vs. 2.87 months for pts with 30 del and wt LMP-1, respectively).
Conclusion: The presence of 30 bp deleted LMP-1 variant, may determine outcome of EBV infected immune-suppressed paediatric LTx pts, such as response to treatment.

P548 Novel mutation (A427S) in the CMV UL97 gene associated with resistance to ganciclovir

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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 antigenaemia 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 (N685S, S655L, N898D, 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.

P549 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-geneTM kit

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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 tranplant 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% 25.5 (n = 5, mean value 23000 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, HHV-6,7,8 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.

P550 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

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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 –20ºC). Two pairs 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 (84.75ºC and 84.75ºC 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 cop/ml (2.65 to 10 log cop/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

essential for the follow-up of treatment by acyclovir, and to understand the pathogenesis of the infection.

P551 Interest of the PCR in the diagnosis of herpes simplex virus oesophagitis

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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 consensus" 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 Al, 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 11 to 3.42×10^6 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 of genome/µg of extracted DNA, 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.

P552 Evaluation of real-time PCR in the detection and management of herpes viruses infections in ulcerative colitis and oesophagitis

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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 evaluation for observation of viral inclusions (VI) and processed with Real-time PCR to detect CMV/HSV (artus 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 exlcuded 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 – 6.9×10^1 copies/ml, VI(+)], CMV [1 man – 1.2×10^5 copies/ml, VI(–)], EBV+CMV [1 man – 3.3×10^5 + 3.1×10^6 copies/ml VI(–)/1 man – 2.2×10^1 + 4.9×10^3 copies/ml VI (+)/1 woman 2.4×10^4 + 1.1×10^3 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, hyperchromaticity and atypical mitoses no viral load was detected. The results in ES were: EBV [1 man – 2.5×10^3 copies/ml (VI+) – 1 woman – 1.6×10^3 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 perfomance of two different diagnostic techniques (histological examination/PCR) seems to optimise the specific diagnosis and leads to an appropriate therapy.

P553 The role of microRNAs in hantavirus infections

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Members of the genus hantavirus (Family Bunyaviridae) cause serious and often fatal human diseases, as haemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in America. Furthermore, in Fennoscandinavia, Puumala virus (PUUV) causes nephropathia epidemica (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 short 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.

P554 Hantaviruses in *Microtus fortis* and *Microtus maximowiczii* in far-eastern Russia

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Background: The reed vole *Microtus fortis* has the highest prevalence of hantaviral 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: *Microtus*-associated hantaviruses circulating in Khabarovsk, 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 cytochrome b gene.
Results: Of 17 hantavirus antigen- or antibody-positive *Microtus* 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, resepectively. 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.

P555 Seewis virus: genetic diversity of a soricid-born hantavirus in Siberia, Russia

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Background: Hantaviral 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 Novosibirsk) 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 0–2% 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 Sorex species. 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.

P556 One tube RT-PCR for Chikungunya and Dengue, including Dengue serotype, with bead-bound probes in a liquid array detected on a Luminex instrument

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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 Qiagen 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 routine singleplex Dengue (SYBR green method on a Light Cycler) 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.

P557 Limited yield of multiplex PCR in adults with acute pneumonia

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Objectives: 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%, Coxsackie/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

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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

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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.

Viral infections: diagnostic and clinical findings

P560 Fulminant deaths caused by Crimean-Congo haemorrhagic fever in Iran

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Objectives: Crimean-Congo Haemorrhagic Fever (CCHF) disease is one of the most important infectious diseases in Iran. This arboviral disease is common between humans and livestock with a mortality rate around 50% whose agent is a virus of the genus Nairovirus and family Bunyaviridae which is transmitted from infected livestock to humans by the bite of Hyalomma ticks or directly between humans. CCHF has been reported from Africa, Eastern Europe, Asia and the Middle East. In addition to clinical signs, the diagnosis of the disease is based on finding anti-CCHF antibodies (IgM and IgG) and a fragment of the viral genome by molecular analysis (RT-PCR) in the patient's sera. In 1999, some cases of clinical findings have been reported from some provinces of Iran, especially the Chahar Mahal – Bakhtiari and according to the studies of the laboratory of Arboviruses and Viral Haemorrhagic Fevers of the Pasteur Institute of Iran (National Reference Lab), the disease is endemic in Iran.
Methods: From 1999 to 2007, 207 sera of human dead cases suspected for CCHF have been sent for diagnosis to the Arbovirus Lab from all around the country, all have been analyzed for the presence of specific antibodies (IgM) against CCHF by ELISA and of the virus genome by RT-PCR.
Results: 64 sera were studied in 207 human dead cases infected by CCHF. In those cases with a molecular diagnostic positive response, in 67.2% of the cases no antibody against CCHF was observed. On the other hand in 9.4% only antibody response without viral genome presence has been observed. In 23.4% of the patients, both criteria of antibody response and molecular diagnosis have been found positive.
Discussion: The results of this study indicate that in a high proportion of fulminant deaths due to CCHF no immunological response has been observed and this can corroborate the virulence of the disease and the resulting mortality rate.
In this regard, for a better analysis of the pathogenicity of the disease, the determination of mediators in the serum (Such as Interleukins . . .) and also the genetical study of intervening factors in the pathogenicity are underway in the different research projects of the laboratory.

P561 Comparative study between the new VIDAS® EBV tests and Liason for the detection of Epstein-Barr virus VCA IgM, VCA/EA IgG and EBNA IgG antibodies in human serum samples

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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 Alkalin Phosphatase. VIDAS EBV VCA 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.

P562 RIDA®QUICK Norovirus: a new dimension for norovirus detection

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Objectives: Noroviruses are commonly associated with large outbreaks in recreational or institutional settings. They are highly infective, thus the rapid and reliable detection of the noroviruses in compromised specimens is of great importance for the hygiene management.
Current approaches such as 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 norovirus.
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 specimen. Its value for a possible prevention of outbreaks in settings where timely reaction are required is certainly indisputable.

P563 First PCR-based detection of a central nervous system infection by Toscana virus in Piedmont region (north-western Italy)

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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 (Nanogen, Italy), and nested rt-PCR for TOSV was performed using the Toscana Virus Oligomix Alert Kit (Nanogen, Italy), that amplifies a 310-bp specific fragment in the S segment of TOSV genome.
Results: The PCRs for Herpes viruses and Enteroviruses 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.

P564 Impact of rapid enterovirus molecular diagnosis in the management of aseptic meningitis in children

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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 suspicious 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 performes in all of them. Forty five (27.87%) had a CSF EV-PCR positive result. Fourteen (26.84%) of CSF samples had no pleocytosis. All of them were patiens <3.6 months (median 1.27 months; range: 0.0–3.6 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.

P565 Efficacy of a direct immunofluorrescence assay versus shell vial culture in the detection of herpesvirus 1 and 2, and varicellazoster in skin infections

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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 varizellazoster virus (VZV) in skin infections.
Methods: The different skin samples were send to the vitrology laboratory in a virus liquid transpotrt medium (MTV, Vircell). For performance of the DFA, 200 µl 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 –20ºC 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 36ºC 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 106 (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 sensitivivility 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 could be the reference method yet, but the molecular techniques have more sensitivity and specificity than the cell culture.

P566 Epidemiological and virological study of aseptic meningitis caused by enterovirus in children

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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 microbiologycal diagnostics 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 evaluate. 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 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.

P567 Multi-centre evaluation of the CMV IgG assay on the Family of Access immunoassay systems from Beckman Coulter

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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 subjects as well as male and female subjects who had CMV IgG testing ordered. The platform utilised was the Access® 2 Immunoassay System. Similar performance has been demonstrated on the UniCel® DxI 800 Access Immunoassay System.
Results: At the cut-off (15 AU/mL), within site total %CV ranged from 6% to 8%. The overall agreement of the Access CMV IgG assay to the VIDAS CMV IgG assay was
99% (95% CI, 97% to 100%). The overall agreement of the Access CMV IgG assay to the AxSYM CMV IgG assay was 100% (95% CI, 98% to 100%).
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.

P568 Toscana virus: serosurvey study in domestic animals within Granada province (southern Spain)

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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 serosurvey study of TOSV in domestic animals within Granada province (south of 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.

P569 Diagnosis of primary rubella infection in 122 adults in Cosenza, Italy and neonatal follow-up

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Objectives: Italy introduced MMR vaccine in 1999, recommending its use in all newborns. Two studies (in 1996 and 2004) found a 10–12% prevalence of seronegativity in women aged 15–39 years, and a 2007 Italian multicentre study found a 65.4% seroprevalence of anti-rubella IgG. In Calabria, occasional infectious episodes after June 2007 culminated in a rubella epidemic in summer 2008.
Methods: Between June 2007 and August 2008, we saw 122 asymptomatic patients (47 males, 75 females, including 20 pregnant women; mean age 21 years, range 5–65) unvaccinated against rubella, who reported evanescent fever, lymphadenopathy, rash (generally regressing from day 2) and joint pain. Biochemical tests revealed normal PCR, ESR, haemochrome; slightly altered transaminases; high LDH. Sera were screened for anti-rubella IgG and IgM by CLIA (LIAISON®, Diasorin Saluggia, Italy) and MEIA (Architect®, Abbott, Germany), and IgG avidity was tested at baseline, and 1 and 2 months after infection. Differential diagnoses included EBV, HHV6, parvovirus B19, Coxsackie and echovirus. Whole blood and urine samples from 8 newborns at birth were tested for specific IgG and IgM, and rubella RNA (Nanogen Advanced Diagnostics, Milan).
Results: The diagnosis was based on IgG/IgM seroconversion and/or confirmed specific IgM and low IgG avidity. Eleven pregnant women infected at 4–12 gestational weeks (gw) underwent voluntary abortions; nine refused prenatal investigation. Four of the eight tested newborns (whose mothers were infected at 15–32 gw) were positive for specific IgM antibodies and rubella RNA in serum and urine (3 asymptomatic; 1 with transitory jaundice, hepatosplenomegaly and high ALT); 4 were negative for both and presumably uninfected. In the newborns and pregnant women, CLIA was more sensitive than MEIA in assaying IgM, thus allowing better definition of acute rubella infection.
Conclusion: The infection in Calabria involved many pregnant women and could have had serious effects on newborns. Our preliminary data show that IgM rapidly decreased, and were absent in 61% two months after the acute phase; IgG avidity rapidly matured in 31% over the same time, and partially matured in the rest. Women should be screened for rubella in the first 8 weeks of pregnancy as delayed testing does not exclude early maternal infection. Biomolecular and serological tests at birth are useful in identifying infected newborns.

P570 The infection of macrophages by enveloped virus

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It was eexperimentally evidenced that macrophages play the important role in resistance to viral infection. In this study, we tested the ultrastructure of infected RNA enveloped (Hantavirus, HV, caused haemorrhagic fever with renal syndrome, and Tick Borne encephalitis virus, TBEV) viruses primary macrophages of mice and monocytes.
The estimate of dynamic accumulation of a viral antigen in cells was made: an indirect method of fluorescent antibodies, the detection of virus RNA was performed with the help of PCR. The definition of TBEV infectious titer was performed on cytopathogenic action on cell culture of SKEC and HV on Vero cells. For the detection of plasma membrane components, a monolayer of infected macrophages was fixed during 1 h in combined composite containing 0.075% ruthenic red. The dehydration of samples was performed in ethanol solution of increasing density and embedded in epon-araldite. The ultrathin sections were prepared in a plane parallel to the cell monolayer.
The viruses adhesion on the cells surface and penetration with reproduction in macrophages from virus-included liquid was determined. The entry and exit of viral particles were realised by local destruction of cells plasmalemma. The type of macrophage infection was autonomous, because the activity of TBEV and HV genome and reproduction of viruses were in cell cytoplasm.
The appearance of virus-inducted structures was indicated in determinate region of cell cytoplasm – fabrics of viral synthesis. The most intensive formation of viroplasts, polyribosomal filaments and microfibrils were identified after 9 hours post-infection by TBEV, and near its the virions formation were determined. The viruses RNA was bound with ribosomes that expressed in form of polyribosomal filaments.
In macrophages infected by HV were detected formation three types of viroplasts in cytoplasm: (1) the compactness viroplasts; (2) viroplasts forming from the first and surrounding by two-layer membrane, (3) lamellar and tube structures. The synthesis of virus nucleoproteins was localised on the surface of first and second type viroplasts, then the

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.

P571 Genotype of varicella zoster virus isolated from Korean elderly patients with herpes zoster

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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 immunosuppresed 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 smaI, 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 were 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+, smaI-, and 1 (4.5%) isolate was pstI– bglI+ smaI– (pOka) genotype.
Conclusion: The seroprevalece of VZV IgG ntibody was high and it was 93.9% in adults. The ifetime prevalence of herpes zoster was 13.7%. The genotype of VZV isolated from adults over 60 years old were all of J genotype.

P572 Prevalence of human papillomavirus types in women with normal citology, atypical squamous cells of unknown significance and cervical intraepithelial neoplasia 1 in Madrid, Spain

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Objectives: The presence of certain high-risk human papallomavirus (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. Citology 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.

P573 Human herpesvirus 8 infection in central Tunisia: seroprevalence among different groups

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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 HHV-8 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.

P574 Variant GII.4 noroviruses in Italian children

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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 GII.4, 14% as GIIb/Hilversum and 5% as GI.1. Phylogenetic analysis of region A and of the ORF1/ORF2 overlapping region of the GII.4 strains recovered in Palermo in the years 2002–2006 revealed the sequential emergence of four variants, GII.4 2002, 2004, 2006a and 2006b. The variant GII.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 GII.4 NoVs in Italian children with gastroenteritis confirms their prominent role as human enteropathogens. At least four distinct GII.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.

P575 Symptomatic Parvovirus B19 infection in immunocompetent adults. Epidemiological, diagnostic, and clinical issues

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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 myelosuppression of all hematological lines characterised 7 cases, while increased serum transaminases were associated in 60% of patients. Three patients were hospitalised (mean 10.8 days of admission), and 4 more cases were followed on Day-Hospital basis (for a median 95-day period); in 3 patients treatment with i.v. high-dosage human immunoglobulins was performed. Elevated levels of specific serum anti-Parvovirus B19 IgM antibodies were detected in all cases. In a 33-year-old female a severe anaemia and a persisting headache, vomiting, and neck stiffness, led to RBC transfusion and a diagnosis of meningoencephalitis, with positive search of IgM antibodies and Parvovirus B19 viraemia (detected by RT-PCR) in the cerebrospional fluid which lasted 3 months, despite treatment with i.v. serum immunoglobulins.
Conclusions: Parvovirus B19 infection may play a significant role also in the adult, immunocompetent subject, and the disease sometimes is not mild and self-limiting, requiring admission and/or frequent outpatient interventions in a significant number of cases. The causes supporting a persistant infection in immunocompetent subjects have not been investigated to date, as well as the pathogenesis of myelosuppression and severe artrhalgia. Symptomatic Parvovirus B19 infection is still an underestimated condition, and therapeutic perspectives are extremely limited.

P576 Epidemiological and clinical characteristics of human parvovirus B19 infections during 2006–2008 in northern Greece

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Objectives: Parvovirus B19 infects children and adults causing erythema infectiosum, polyarthritis, aplastic crisis in patients with chronic haemolytic anaemia, rash, fever, and foetal anaemia-related disease or foetal death.
The aim of this effort was to study acute human parvovirus B19 infections during the years 2006–2008 in northern Greece on their epidemiological and clinical aspect.
Methods: Specimens were obtained from 56 patients suspected for acute h. parvovirus B19 infection (17 in 2006, 29 in 2007, and 10 in 2008). 27 of them belonged to children (1 day – 15 years old) and 29 to adults (17–65 years old). The infection was proved by PCR amplifying a 227 bp DNA segment, part of the viral gene VP1, in whole blood and in two cases in pleural fluid. Detection of specific IgM antibodies in the patients' blood serum was performed by ELISA.
Results: 19 out of the 56 specimens were found to be positive by PCR (33.9%): 3/17 (17.6%) in 2006, and 16/29 (55.2%) in 2007, $p = 0.03$. Specific IgM antibodies were detected in all 19 positive patients. 10 positive specimens belonged to children and 9 to adults, $p = 0.64$.
Children presented with haematological disorders, mainly types of anaemia (six cases), hydrothorax/ascites (two cases), arthritis (one case), and liver transplant rejection (one case). Adults presented with pregnancy complications (two cases), arthralgia/arthritis (three cases), febrile syndromes (three cases, two of them on SLE and one on chronic haemoglobinopathy background), and atypical rash (one case).
Conclusions: An annual variation in the circulation of h. parvovirus B19 was observed, presenting a statistically significant increase of acute infections during 2007 in northern Greece.
Children and adults were equally affected, however differences in clinical manifestations were observed between them, with haematological dysfunctions predominant in childhood.

P577 Distribution of genotypes of human papilloma virus in women from different countries

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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 an 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 (29.8%) 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.

	VPH6	VPH11	VPH16	VPH18	VPH31	VPH33	VPH45	VPH58	VPH NT	Coinfection
Brazil	8.78	11.49	25.68	9.46	14.86	6.76	2.03	10.81	37.16	18.92
Latin America	11.46	9.38	13.54	8.33	10.42	7.29	1.04	10.42	53.13	7.29
Spain	30.67	17.33	24	1.33	12	2.67	0	8	34.67	12
Eastern Europe	30	10	15	5	5	15	0	10	40	25
Africa	9.09	9.09	27.27	9.09	9.09	0	0	0	45.45	9.09

P578 Seroepidemiology of Varicella zoster virus infections in Greek adults during a two-year period

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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-ALPHADIA-BELGIUM).
Results: The seroprevelance 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 for varicella was 0.76% (7/918) and for zoster 1.3% (12/918). IgM antibodies were detected in all patients with varicella and in 2 patients with HZ. The remaining 10 patients with HZ developed only IgG and IgA antibodies.
Conclusions: During the acute phase of HZ infection, IgA antibodies are usually developed, while IgM antibodies are often absent. Although varicella and HZ 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.

P579 Clinical and genetic analysis of human bocavirus in children with lower respiratory tract infection in Taiwan

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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 amplifyed and sequenced to re-construct their phylogenetic trees.
Results: HBoV was detected in 30 (5.6%) 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.
Conclusion: HBoV might have circulated in Taiwan for a certain period and is involved in the aetiological agents responsible for lower respiratory tract infection in children. Evolutionary networks suggest that HBoV might have an opportunity for interbreeding of virus and genetic recombinant among the different genes.

P580 Activation of interferon response in human PBMC by avian influenza H5N1 virus

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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-a and -g 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-a and -g 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 -g 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
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.

P581 Protein C, protein S levels and other haematologic parameters in patients with Crimean-Congo haemorrhagic fever

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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, fibrinojen 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 on 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

n: 50	Protein C	Protein S	Factor VIII	Fibrinogen	D-dimer	PT	aPTT	INR
Normal, n (%)	42 (84)	23 (46)	48 (96)	40 (80)	2 (4)	42 (88)	39 (78)	40 (80)
High/Prolonged, n (%)	–	–	–	2 (4)	48 (96)	8 (16)	11 (22)	10 (20)
Low, n (%)	8 (16)	27 (54)	2 (4)	8 (16)	–	–	–	–

Conclusion: Protein C and protein S consumption may be increased and/or syhnthesis 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.

P582 Analysis of the importance of nuclear export of the influenza A virus NS1 protein for viral replication

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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 cytokine production of infected A549 cells was measured by ELISA.
Results: 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.

P583 Prevalence of human papillomavirus types in women with cervical intraepithelial neoplasia in Madrid, Spain

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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 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: 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

P584 Tuberculous meningitis in adults: evaluation of 38 cases

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Objective: Tuberculous meningitis (TM) as a marker of uncontrolled of 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 $\pm$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%), wheras 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/mm^3, 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 sequela 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 melitus, 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.

P585 Genitourinary tuberculosis: a 12-year experience

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Background: We present our experience with genitouritary 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 days, 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 noted and the urinary tract system was normal in 24 patients at the last evaluation.
Conclusions: GUTB has a wide spectrum of clinical and radiological findings. A high clinical suspicion is warranted for diagnosis. MGlT 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

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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 Daneshvari 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

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Objectives: The incidence of oral tuberculosis, observed mainly secondary to pulmonary infection has been diminished to less than 1% following the succesful 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. Oral tuberculosis is found secondary to a pulmonary lesion in 42% of the cases, to extrapulmonary TB in 3.5% and in all the remaining cases is found primary. Antituberculous treatment shows effectiveness in (%) of cases, while 3/144 deaths are documented due to the spread of the mycobacterial infection in sites such as the meninges, or due to extensive pulmonary (miliary) disease.
Conclusions: The investigation for a mycobacterial infection in oral cavity lesions should be intense in the dental office due to the re-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

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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 disorientated. 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 microti*: description of 6 recent cases in France

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Objectives: *Mycobacterium microti* 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. microti* has been reported in 1998. Here, we report the clinical, microbiological and molecular characteristics of 6 human pulmonary tuberculosis due to *M. microti* in France.
Methods: We carried out a retrospective study of cases diagnosed between 2000 and 2007. The isolation of *M. microti* was performed by culture from respiratory samples and the isolates were identified as *M. microti* 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, sputum microscopic 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. Drug susceptibility testing revealed sensitivity to isoniazid, rifampin, ethambutol and streptomycin. Treatment regimens are categorised into two groups; 3 patients were treated with tri-therapy (rifampin, isoniazid, and pyrazinamid), 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. microti* 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

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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 specimens 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 active 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

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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*, *M. shottii* and *M. pseudoshottii* being the species most frequently isolated. 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 parasitological, virological and bacteriological investigations.
Results: At gross examination ulcerative cutaneous lesions, yellow mucus 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-Neelsen 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*. Parasitological 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.

P592 Particularities of tuberculosis in teenagers horizontally infected with HIV-1 during infancy

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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: retrospectively study (01.01.2002–31.12.2007); we have evaluated 415 teenagers (born1988 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/mm^3 while the average viral load has been 5.6±5.4 log copies/ml. Relatively 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%) 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 tuberculostatics treatment.

P593 Non-tuberculous Mycobacteria: incidence and clinical significance

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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 sensitivity 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.

P594 Prevalence of *Mycobacterium tuberculosis* drug resistance in a Spanish teaching hospital during a six-year period

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Objective: 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 MGIT 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 MGIT 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 immunodeficiency 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.

P595 *Mycobacterium genavense* infections: a French national multi-centre retrospective study from 1996 to 2007

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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

form. The mycobacterial DNA was sequenced to identify potential sequence variants within the ARNr16 s, hsp65 and rpoB genes.
Results: Twenty five cases of *M. genavense* were identified in 15 centres. Twenty patients were AIDS patients, 3 had a solid transplantation and 2 had a sarcoidosis. Sixteen patients (64%) were male, mean age was 42 years (SD=10) and the median CD4 count was 13/mm^3. Twenty four patients had a disseminated infection with fever (75%, n = 18), weight loss (79%, n = 19), abdominal adenopathies (62.5%, n = 15), abdominal pain (71%, n = 17), diarrhoea (62.5%, n = 15), splenomegaly (71%, n = 17), hepatomegaly (62.5%, n = 15). *M. genavense* was isolated in the adenopathies (n = 13), digestive biopsies (n = 9), blood (n = 6), sputum (n = 3), stools (n = 3), bone marrow (n = 5). Eleven patients (44%) died, 8 (32%) were cured and 6 (24%) had chronic symptoms. The one-year, three-year and five-year survival rates were respectively 72%, 59% and 53%. Four sequence variants of *M. genavense* were identified within the hsp65 gene.
Conclusion: The prognosis of *M. genavense* infection has dramatically improved with HAART. We identified a genetic polymorphism of the mycobacteria useful to improve the molecular diagnosis of the infection.

P596 Extensively drug-resistant tuberculosis in patients recently immigrated from Eastern Europe. Microbiological, therapeutic, and public health features

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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 6 y 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 technologic 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.

P597 Significant re-emerging of tuberculosis in Italy. Relationship with potential risk factors, and comparison between native residents and foreign immigrants

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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 of 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 pleuropulmonary 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 predominatly 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.

P598 Trends of tuberculin skin test positivity rate among children 6–14 years old in Attica, Greece

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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 >10 mm) of schoolchildren, in the catchment area of the public primary health-care centre of Vari, 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.

P599 **Comparison of smear positive pulmonary tuberculosis in young adult and elderly patients**

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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.

P600 **Application of the optimal 24 MIRU-VNTR loci set for *Mycobacterium tuberculosis* strains improves the correlation between strain typing and epidemiological data**

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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 aims to improve the concordance of strain typing data with epidemiological data has been published. In the Midlands region of the UK, all *M. tuberculosis* isolates are typed using 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 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 MIRU-VNTR loci set 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.

P601 **Comparison of *Mycobacterium tuberculosis* strains prevalent in the Indian Sub-continent and the UK**

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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 TbD1 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 TbD1 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 = 8), Haarlem (n = 4), Beijing (n = 3), and AFRI/S/U/X (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 TbD1 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 933/935 being modern (M24 < 2 repeats).
Conclusion: Previous studies have indicated that *M. tuberculosis* strains present 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.

P602 **Novel mutations outside rifampin resistance determining region associated with rifampicin resistance in *Mycobacterium tuberculosis***

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Background: More than 90% of rifampicin resistance in *Mycobacterium tuberculosis* was shown to be caused by mutations inside the 81-bp rifampin resistance determining region (RRDR) located in the centre of the 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

a mycobacterial expression vector pVV16 and were transformed into *M. smegmatis* MC^{2155} for rifampicin minimal inhibition concentration (MIC) study.
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 habour any mutation in other region of rpoB. Transformation of the mutated rpoB gene into *Mycobacterium smegmatis* MC^{2155} rendered an increase in MIC from 32 ug/ml to 128 ug/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 rifampcin-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.

P603 High-resolution melting analysis for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates

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Objectives: Rapid detection of drug-resistant tuberculosis improves therapy and transmission control. We used high-resolution melting (HRM) curve analysis, which detects single nucleotide polymorphisms, to identify rpoB, katG and inhA mutations responsible for rifampin (RIF) and isonizid (INH) resistance.
Methods: Total 161 clinical isolates that included 65 INH-resistant (R), 61 RIF-R, and 76 dual-susceptible (S) strains were tested. The PCR primers amplified the region containing codons 510–528 of rpoB, codon 315 of katG, and nucleotide substitution C209T in inhA. The melting curves were obtained with fluorescence of Eva Green dye by using Corbett Life Science Rotor-GeneTM 6000 instrument. All R strains and randomly selected 40 S strains were subjected to DNA sequence analysis.
Results: All 96 INH-S strains showed unique melting curves in the katG and inhA PCR (specificity 100%). Sixty-two INH-R strains had melting curves different from wild-type strains. Sequence analysis showed 48 katG S315T and 22 inhA C209T mutations. Three INH-R strains showed wild-type melting curves (sensitivity 95.4%). All 100 RIF-S isolates displayed a single peak in rpoB PCR (specificity 100%). Among 61 RIF-R strains, 53 had distinct mutant-type melting curves, demonstrating the D526Y mutation in 8 and the S531L mutation in 44. Three strains with D516Y mutations exhibited melting curves similar to the wild type, but a difference plot curve demonstrated a distinction. The remaining strains had 1 D516V and 4 H526D mutations in rpoB (sensitivity 91.8%).
Conclusion: An HRM analysis is rapid and detects RIF- or INH-R *M. tuberculosis* with high sensitivity and specificity.

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

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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 prodrug 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-plasmid 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

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

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.

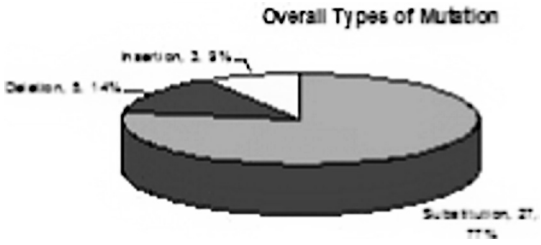

Figure 1. Frequency and types of pncA mutation in South Korea.

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

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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 Lisboa 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.

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

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Objectives: Streptomycin (STP) was the first antibacillary drug introduced in the treatment of tuberculosis in 1944. With the development of further antibacillary 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 rRNA 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 10/57 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 susceptibity testing. The independent occurrrence suggests however, that the acquisition of such mutations present an adaptative advantage.

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

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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.

P608 Binding domain of the TMC207 in subunit C of mycobacterial ATP synthase

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Objectives: In mycobacteria, the subunit c of ATP synthase, encoded by atpE, has been identified as being the target of the new anti-*tuberculosis* drug R207910 (TMC207) (Andries, Science, 2005). Here, we report on the in vitro isolation of reference strain (H37Rv) and clinical strains of *Mycobacterium tuberculosis* mutants resistant to TMC207 and the characterisation of amino acid substitutions in AtpE. We also evaluated the level of resistance conferred by these mutations in *Mycobacterium smegmatis* and we generated a model for the structure of the ring of c subunits in *M. tuberculosis* to predict the role of these residues in the binding of the drug by docking experiments.
Methods: Mutants were obtained by selection on 7H11 + OADC agar medium containing TMC207 at various concentrations. Mutations in the atpE gene were identified by PCR and sequencing. The plasmid pLYG204 was used to complement *M. smegmatis* mc2155 with atpE gene carrying the mutations identified in the resistant strains. The 3D model was made using data from the crystal structure of the c ring of

Ilyobacter tartaricus (Meier, Science, 2005) and Autodock was used for docking experiments.
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.

P609 Molecular epidemiology and antimicrobial susceptibility of *Mycobacterium abscessus* isolated from patients with cystic fibrosis

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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 CF 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

Interpretation	Antimicrobial agent							
	CLA	LZD	TOB	FOX	AMI	SMX	DOX	CIP
Susceptible (n)	35	21	5	6	26	0	4	1
Intermediate (n)	0	11	14	19	3	0	5	1
Resistant (n)	0	12	16	9	6	35	26	33

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.

P610 Genetic characteristics of *Mycobacterium tuberculosis* strains, isolated among Moscow residents

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Objectives: Moscow is the largest city of Russia with intensive migration flow (including peoples from Former Soviet Union (FSU) countries). High TB morbidity and increasing numbers of multidrug-resistance (MDR) in Russia and FSU countries call an attention to the effective preventive, prophylaxis and surveillance measures, aimed to prevent and avoid *M. tuberculosis* transmission and MDR spread in the community. The purposes of this study were to evaluate epidemiological diversity and MDR spreading among *M. tuberculosis* strains, circulated among Moscow residents.
Methods: *M. tuberculosis* strains (n = 115) have been randomly selected from sputum of epidemiologically unrelated TB patients, which were Moscow residents. Species identification and drug susceptibility testing to main antituberculosis drugs – rifampicin (RIF) and isoniazid (INH) – were performed according to the WHO recommendation protocols. Standard procedures of spoligotyping and 24-loci VNTR typing were carried out as described previously. For detect specific antimicrobial resistance SNPs minisequencing reaction followed by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) of the reaction products was used.
Results: Among 115 *M. tuberculosis* strains 76 (66.0%) were identified as MDR, 20 (17.4%) were susceptible to RIF and INH, and 1 (0.9%) and 18 (15.7%) were resistant either to RIF or INH, respectively.
Mutations in the RRDR of the rpoB gene were detected in 64 (64/77; 83.1%) RIF resistant strains. The Ser531Leu substitution was prevalent among them (49/64; 76.5%). Aberrations in the Ser315 codon of katG and/or in the inhA promoter region were found in 79 (84.0%) of 94 INH-resistant strains.
Strains belonged to the Beijing family were prevalent – 66.0% (76/115), the other two large families (LAM and T) occurred rarer (10.4% and 8.7%, respectively). Seventy one different VNTR profiles were identified. Tree main 24-loci VNTR clusters included 34 (34/115; 29.5%) strains belonged to Beijing family. MDR-phenotype revealed more frequently among Beijing strains (59/76; 77.6%), than among other strains (17/39; 43.6%) (χ-square = 13.3; p = 0.0003).
Conclusion: Obviously, Beijing family plays a significant role in the spread of MDR TB in the Moscow. Spoligotyping and 24-loci VNTR typing combination demonstrate sufficient discrimination power and may be useful for TB monitoring in Moscow region.

P611 Detection of efflux pump genes among clinical isolates of non-pigmented rapidly growing mycobacteria and their relationship with phenotypic resistance

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Objectives: To detect the presence of previously described efflux pumps genes (lfrA for quinolones and tap for tetracyclines) among clinical isolates of non-pigmented rapidly growing mycobacteria (NPRGM), and to correlate its presence with the in vitro susceptibility results.
Methods: In vitro susceptibility of clinical isolates of NPRGM was studied using the reference microdilution technique. Breakpoints to determine susceptibility of the strains were those described by the CLSI. Tested antibiotics were Doxicycline (DOX), Tigecycline (TGC) and

Ciprofloxacin (CIP). Gene detection was performed by PCR analysis using primers designed for each gene. Accuracy of detection was confirmed by sequencing of the amplified products. We use as controls the type strains of *M. smegmatis* (for lfrA) and *M. fortuitum* (for tap).
Results: 166 clinical isolates of NPRGM were included in the study (9 *Mycobacterium abscessus*, 30 *M. chelonae*, 89 *M. fortuitum*, 5 *M. mageritense*, 7 *M. mucogenicum*, 22 *M. peregrinum*, 2 *M. alvei*, 1 *M. septicum* and 1 *M. porcinum*). Only 4 strains were positive for lfrA (2 *M. chelonae*, 1 *M. fortuitum* and 1 *M. mucogenicum*). Two of these strains (1 *M. chelonae* and *M. mucogenicum*) were resistant to ciprofloxacin (MIC: 4 mg/L), while the other two were susceptible (MIC: 0.12 mg/L). For tap, 109 strains were positive: 3 *M. abscessus* (33.3%), 12 *M. chelonae* (40%), 75 *M. fortuitum* (84.3%), 2 *M. mageritense* (40%), 15 *M. peregrinum* (68.2%), 1 *M. alvei* and 1 *M. porcinum*. The results of MIC90 (mg/L) and non-susceptible percentages against DOX and TGC for each subgroup are shown in the table for strains with $\geqslant$5 isolates (NS: non-susceptible).
No strain of *M. mucogenicum* was tap positive. One strain of *M. alvei* and the strain of *M. porcinum* were also tap positive. No differences between tap positive and tap negative strains were observed for resistance against DOX (Fisher's exact test, p = 0.055).
Conclusions: lfrA efflux pump is rare among clinical strains of NPRG. On the contrary, tap gene is common among these mycobacteria, although its relationship with DOX resistance is not clear, and it has no effect on susceptibility against TGC.

Species	*tap+*			*tap–*		
	MIC_{90} DOX	% DOX NS strains	MIC_{90} TGC	MIC_{90} DOX	% DOX NS strains	MIC_{90} TGC
M. abscessus	>64	0	1	>64	100	0.5
M. chelonae	32	41.7	0.25	64	77.8	0.5
M. fortuitum	64	68	0.25	64	64.3	0.5
M. mageritense	8	50	0.12	16	66.7	0.06
M. mucogenicum	–	–	–	64	57.1	0.5
M. peregrinum	32	53.3	0.06	64	100	0.12
All strains	64	63.3	0.25	64	77.2	0.5

P612 Incidence, clinical and epidemiological risk factors, and outcome of drug-induced hepatitis due to anti-tuberculous agents in new TB cases

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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 the 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 10.26±5.95 (Median = 9; 0–32 days).
Anorexia, nausea, vomiting, abdominal pain, jaundice, diarrhoea, decreased 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).
After adjusting with logistic regression, all of the anticipated factors retained the statistical significance.
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.

P613 Evaluation of VersaTREK® system for isolation and susceptibility testing of *Mycobacterium tuberculosis*

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The increasing number of new tuberculosis cases and emergence of drug resistant strains of *M. tuberculosis* (MTB) is of public health concern. To adequately address this threat requires reliable microbiological methods to both isolate this organism and perform susceptibility testing. The VersaTREK®(TREK) system detects positive cultures by measuring changes in gas pressure whereas the BacT/ALERT 3D®(BioMérieux) system detects pH changes.
Objective: To evaluate the VersaTREK®(VTK) system and compare it to the Bact-Alert 3D®(BA) system for the isolation of mycobacteria from clinical specimens and for susceptibility testing of MTB.
Methods: For a period of two months all samples received in the Microbiology Laboratory of Hospital Parc Taulí (Sabadell, Spain) requiring mycobacteria culture were processed on BA and VTK systems. The samples were decontaminated, inoculated into each vial and incubated for 42 days following standardised methods. Cultures confirmed to contain acid-fast bacteria growth were identified from bottles using Mycobacteria Genotype CM kit®(Hain).
Susceptibility testing was performed in both systems on non-duplicate MTB isolates from the processed samples and a group of archived drug-resistant MTB. Isoniazid (H), rifampin (R), streptomycin (S), ethambutol (E) and pyrazinamide (Z) were tested following standardised protocols. An additional 10 WHO National Reference Laboratory strains were tested in the VTK system.

Table 1. Susceptibility testing results comparing BacT/ALERT-3D® and VersaTREK® systems

Drug and result category	No. of isolates by result category and system used	
	BacT/ALERT-3D®	VersaTREK®
Isoniazid (Low – 0.1 ug/mL)	22	22
Susceptible	9	9
Resistant	13	13
Isoniazid (High – 0.4 ug/mL)	Not tested	22
Resistant		11
Rifampin (1 ug/mL)	22	22
Susceptible	21	21
Resistant	1	1
Ethambutol (Low – 5 ug/mL)	22	22
Susceptible	21	20
Resistant	1	2
Ethambutol (High – 8 ug/mL)	Not tested	22
Resistant		2
Streptomycin (Low – 2 ug/mL)	22[a]	22
Susceptible	21	21
Resistant	1	1
Streptomycin (High – 8 ug/mL)	Not tested	21
Resistant		1
Pyrazinamide (300 ug/mL)	22[b]	22
Susceptible	21	21
Resistant	1	1

[a] 1 ug/mL; [b] 100 ug/mL.

Results:
a. Isolation. During this two month period, 327 specimens were received for mycobacterial culture. Of these 28 (8.6%) were positive. Twenty-five MTB and one *M. intracellulare* were isolated in both systems. The average time to detection was 8 days in both VTK and BA. In

addition, one isolate each of *M. xenopi* and *M. scrofulaceum* were isolated only in VTK. The VTK had 4 (1.2%) false positive cultures while the BA system recorded 28 (8.6%). The contamination rate was 3.7% in both systems.

b. Susceptibility testing. A total of 22 MTB isolates were studied. Briefly, the correlation was 100% for both systems for H and R resistant strains. Detailed results on Table 1. The VTK allowed obtaining two points of MICs for H, E and S.

The result of the WHO quality control strains showed a good performance of VTK system.

Conclusion: The VTK system isolated two additional strains of mycobacteria and had a lower false positive rate than the BA system and proved to be a reliable system for the isolation of mycobacteria and susceptibility testing of MTB.

Clinical epidemiology of nosocomial infections

P614 Do splenectomized patients for trauma suffer from more early postoperative infections?

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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 2006 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 secundary 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 [20–31.2] vs 25.5 [19.2–29]; p = 0.84). Pneumococcal 23-v (mean = 0.44 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 *S. aureus* 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.

P615 Early intravenous to oral antibiotic switch therapy is effective in the treatment of infected total hip replacement

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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.

P616 Do cardiac centres in the United Kingdom have a standard policy for antibiotic prophylaxis in cardiac surgery? First clinical audit in last 10 years

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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 Lancashire cardiac 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 cardiac 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 3 (12.5%). The clostridium difficile infection rates per month (23/26 responses) are <1% in 14 (60.9%) 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.

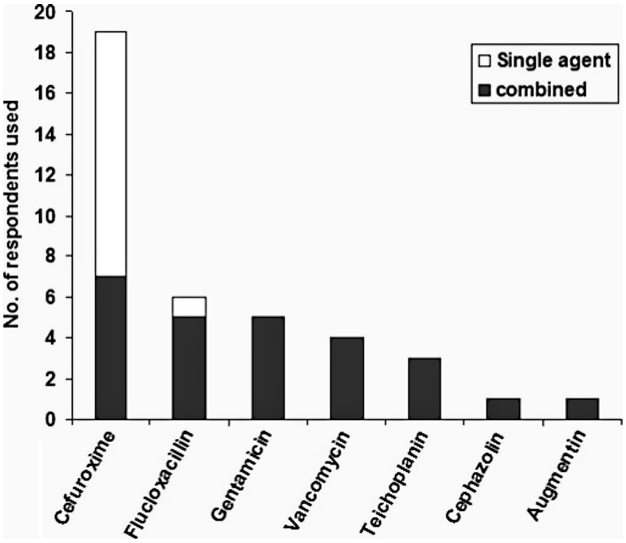

Figure: Prophylactic antibiotic usage in cardiac surgery.

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 35ºC 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.

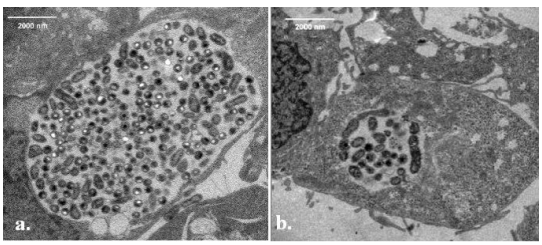

Figure 1. Antimicrobial consumption trends in DDD per 100 bed-days, Colombia, 2002–2007.

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)

[1] Eisenberg M J et al 2006 Am J Cardiol 98:375–382.
[2] Hayashida K et al 2002 Heart Vessels 16:137–145.
[3] Neumann F et al 2001 Lancet 357:2085–2089.

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

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Objectives: 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%).

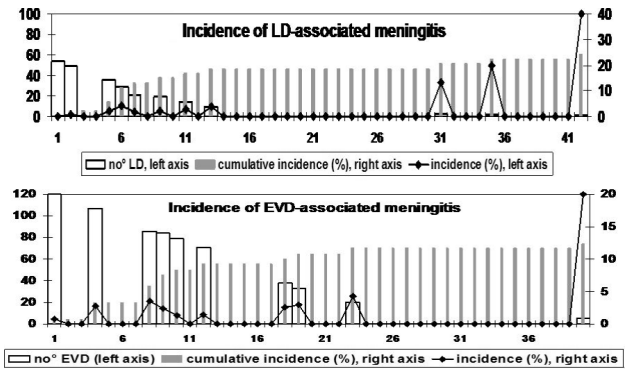

Figure 1. Incidence of LD- and EVD-associated meningitis.

Meningitis was more often in patients with SAB ºV (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 neoplasm (10/28; 36%; OR: infinity) and in patients with LD versus EVD (OR: 2.06). Association with SABºV ($p = 0.005$) and neoplasm ($p = 0.000$) 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.

P619 Ventricular assist device-related infections

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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 septicaemia 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 HT-VAD unit to prevent and manage device related infections in the MDR pathogen era.

P620 Risk factors for colonisation by colistin-resistant *Klebsiella pneumoniae* in critically ill patients

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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 Etest 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 colRKP 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.

P621 Nationwide investigation into the prevalence of nosocomial infections in Belgium

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Objectives: Since the prevalence of nosocomial infections (NI) in Belgium had not been determined nationwide since 1984, the Federal Ministry mandated the Belgian Healthcare Knowledge Center to set up a national study in 2007 in order to estimate the actual burden of NI in Belgium.
Methods: A point prevalence study (PPS) was conducted in collaboration with the Federal and regional platforms for hospital hygiene during a 4 week period (Oct 15 – Nov 11, 2007), involving 17,343 patients from 56% of all Belgian acute care hospitals excluding long stay psychiatric care and day care. A specific software comprising an expert system establishing the diagnosis of NI according to the CDC-definitions was developed in order to enable bed-side registration of symptoms instead of diagnoses. NI appearing after discharge were not studied.

Prevalence of nosocomial infections registered in the Belgian national surveillance study 2007

Bed category	Prevalence of patients with NI	Type of NI	Prevalence	Proportion of all NI
Adult ICU	25.3%	Urinary tract	1.69%	23.9%
Neonatal ICU	12.6%	Lower respiratory tract	1.42%	20.1%
Chronic care	7.6%	Surgical site	1.04%	14.6%
Geriatric	7.4%	Bloodstream	0.96%	13.6%
Surgical	5.9%	Gastrointestinal	0.88%	12.5%
Non-intensive neonatal	5.6%	Ear, Eye, Mouth	0.18%	2.6%
Medical	5.2%	Bone and Joint	0.15%	2.1%
Paediatric	2.7%	Upper respiratory tract	0.11%	1.5%
Psychiatric	1.7%	Reproductive tract	0.07%	1.0%
Maternity	1.0%	Central Nervous System	0.05%	0.7%
All	6.2%	Cardiovascular system	0.04%	0.6%

Results: After correction for sampling ratio in different hospitals, the prevalence of patients infected nationwide was 6.2% (CI95% 5.9–6.5) and the prevalence of NI is 7.1% (CI95% 6.7–7.4). Break-downs according to bed-category and type of NI are shown in the table.
Surgical site represented 38.7% of NI in patients in surgical wards. Infections of the urinary, bloodstream and lower pulmonary tract accounted for 23.6%, 22.8% and 20.4% of NI resp. in patients in medical

wards. In adult IC units, pneumonia and septicaemia 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

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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 of 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 precautions was widely variable. In many hospitals, several essential IC measures were not incorporated in local regulations and in spite of this, compliance is average or low and systematic monitoring almost non-existent.
Although many hospitals formerly claimed to perform POWI surveillance, the survey demonstrates that standardised surveillance projects with follow-up and feedback to surgeons is generally lacking.
Conclusions: This investigation confirms the extensive variability of IC practices in Belgian OS, both with respect to standards described in local operating procedures as to actual compliance with local and/or internationally prescribed IC measures. National IC standards for OS are urgently needed, truly implemented and officially monited. The variability in approach of IC and the extensive lack of POWI surveillance projects demonstrate the necessity for Belgian IC professionals to practice process surveillance in OS rather than outcome surveillance.

P623 Evaluating the impact of the National Resource of Infection Control

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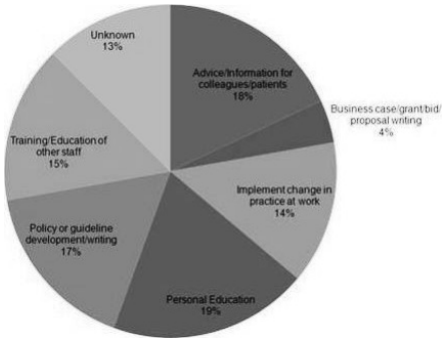
Objectives: The National Resource for Infection Control (NRIC) became a member of the International Federation of Infection Control in 2008 to support a growing number of international users. The need to evaluate the impact of NRIC on professionals in the infection prevention and control field is essential to identify how successful it is and where future investment can be targeted and an impact evaluation was undertaken in Spring 2008. Participants were recruited by an advert on the NRIC website, through the NRIC mailing list (approximately 1800 members) and via the Infection Prevention Society (IPS) website forums and contacts.
Methods: The impact evaluation was conducted using the Impact-ED model for digital library evaluation. This provides a set of criteria around 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

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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

the workload (patient-based data for all patients, for infected patients only, or aggregated infection and denominator data), type of risk factors, exclusion or not of specific types of departments (e.g. paediatrics), types of hospitals (e.g. long-term care), types of patients (e.g. minimum length of stay), type of investigators (internal vs external) and their training, case finding methods, the use of data sources such as a pharmacy or microbiology database and the type of microbiological data collected during the PPS.

Conclusions: The European Centre for Disease Prevention and Control (ECDC) plans an EU-wide PPS of NI in order to obtain nationwide data on all types of NI from all EU Member States. The current review however shows that most MS may have to adapt their current national protocol in order to comply with an EU-agreed method including at least common case definitions, comparable case ascertainment and a minimal common dataset for stratified comparisons.

Methodological difference	%	Countries[a]
Case definitions		
Diagnostic related groups	11.8%	LV, SE
CDC, modified	11.8%	FR, NL
CDC, unmodified	76.5%	Other
Imported HCAI included[b]	47.1%	DK, ES, FI, FR, IE, NL, SE, UK
Included infections		
All infections	52.9%	BE, GR, IT, LT, LV, NL, PT, SE, SI
Only main infection types	11.8%	NO, DK
Exclusion of secondary bloodstream infections	23.5%	UK, IE, FI, DE
Exclusion of asymptomatic bacteriuria	11.8%	ES, FR
Data collection type/workload		
Aggregated numerator and denominator	11.8%	NO, DK
Patient-based numerator and aggregated denominator	11.8%	SE, LV
Patient-based numerator and denominator	76.5%	Other
Exclusion of specific patients or specialties	17.6%	FR, NL, FI

[a] BE: Belgium, DE: Germany, DK: Denmark, ES: Spain, FI: Finland, FR: France, GR: Greece, IE: Ireland, IT: Italy, LT: Lithuania, LV: Latvia, NL: Netherlands, NO: Norway, PT: Portugal, SE: Sweden. SI: Slovenia, UK: United Kingdom; references: see review in ECDC Annual Epidemiological Report on Communicable Diseases 2008 available from http//ecdc.europa.eu, except Belgian PPS available from www.kce.fgov.be.

[b] Not always included in main HCAI prevalence result; UK, IL: only if re-admission from same hospital.

P625 A national point prevalence measurement of healthcare-associated infections in somatic care in Sweden, 2008

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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 of internal medicine, 18.2% to surgery and 10.4% to orthopedics. 2,227 patients with 2,385 diagnoses of HAI were recorded. The most frequent HAI diagnoses were infections of the lower urinary tract, 20.5% (68% device related), skin and soft tissues 16.5%, lower respiratory tract 14%, fever 7.7%, septicaemia 6%, contagious gastrointestinal infections 5.9%. The overall HAI prevalence was 11.1%. Large variations of HAI were observed between different hospitals; tertiary hospitals 7.4–19.8%, secondary hospitals 6.2–15.1% and primary hospitals 2.8–23.8%. The risk factors for HAI was central venous lines, present in 12.8% of all patients, catheter á demeur in 21.9%, immuno-suppression in 8.4%, surgery 30.8%, mechanical ventilation 2.1%. 4.4% of the patients were treated in an ICU. 32.7% were treated with antibiotics and 9.7% were 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.

P626 Epidemiology and outcome of *C. difficile* infection in a large UK teaching hospital

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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 38% 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.

P627 English voluntary surveillance scheme for *Clostridium difficile* infections: seasonal variation by age group (January 2000 – December 2008)

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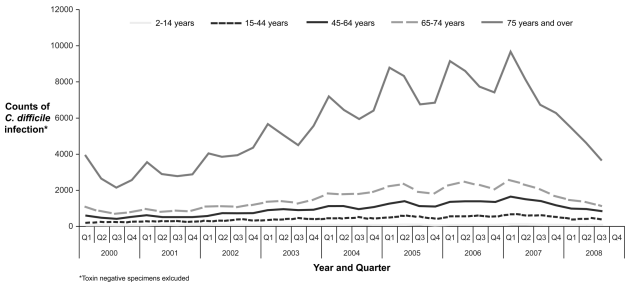
Objectives: This paper describes apparent age variation in seasonality of *C. difficile* infection (CDI) in hospital and community associated cases in England.

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, soundex, age and specimen date.

301,797 cases of CDI where the patient's age was known were reported (January 2000 to September 2008). Patient age at the time of diagnosis was used to stratify age groups as: 2–14 years, 15–44 years, 45–64 years and ⩾75 years. Data on patients aged less than 2 years of age were excluded from the analysis (1,720 reports). Also excluded from the analysis were specimens without age data (5,726 cases) and reports stated as toxin negative (4,723 cases).

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.



P628 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

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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 type 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 tandem repeat analysis (MLVA).

Results: During the study period, 168 patients were included, 57 (34%) of whom had CDI with type 017, 46 (27%) with type 027, and 65 (39%) with other types. Controls consisted of 77 non-CDI diarrhoeal patients and 162 patients without diarrhoea. Independent risk factors for CDI were nasogastric intubation (OR 2.7), recent admission (OR 2.1), use of second generation cephalosporins (OR 12), clindamycin (OR 11) and antifungal agents (OR 8.1). Individual comparison of CDI due to types 017 and 027 with CDI due to other types, identified age above 65 years as risk factors for types 017 and 027 (OR 4.6 and 6.1, respectively) and use 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 diarrhoeal and non-diarrhoeal 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

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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%.

Therapy groups	No. of patients		Bloodstream infection				Pneumonia			
			Rate (%)		Incidence density[a]		Rate (%)		Incidence density[a]	
	AML	ALL	AML	ALL	AML	ALL	AML	ALL	AML	ALL
All	768	244	16.0	11.9	9.3	9.7	9.1	7.0	5.3	5.7
Standard	345	161	15.1	11.2	8.3	10.2	9.3	2.5	5.1	2.3
Extended	100	47	18.0	4.3	13.2	3.5	5.0	10.6	3.7	8.7
Relapse	25	4	28.0	n/a[b]	12.9	n/a[b]	28.0	n/a[b]	12.9	n/a[b]
>60 years	291	32	15.5	28.1	9.1	15.0	7.9	25.0	4.6	13.3

[a]No. of infections/1,000 neutropenic days. [b]n/a.: not applicable due to small group size.

Conclusions: The ONKO-KISS_AL infection rates and incidence densities appear similar to those in patients with allogeneic bone marrow or peripheral blood stem cell transplantation derived from ONKO-KISS. With ongoing surveillance, variation among centres stratified for case-mix can be estimated and correlated with infection control and antibiotic policy practices.

P630 Incidence of infections in burn centres in France

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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 y.o [16–52], sex ratio (M/F) 7/10, Total Burn Surface Area (TBSA) 10% [5–20]. 24% are younger than 15 y.o 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 patients is 1.7 episodes/100 patient-days and density incidence of infections is 2.0 episodes/100 patient-days. The site of infection and their relative rate are burn wound (32%), lung (32%), urine (17%) and bacteraemia (14.6%). Multi-site infections represent 14.6% of all cases. Gram-negative pathogens are the leading strains (72%) with *Pseudomonas aeruginosa* (28%), *Proteus* (18%), *E. coli* (16%), *Serratia* (11%), *Klebsiella* (4%). Gram positive pathogens represent 55% of all infections with *Staphylococcus aureus* (32%) and Enterococci (9%). Yeasts represent about 5%. Strains antibiotic susceptibility analysis shows that 27% of them are multi-resistant. Overall mortality is 5%.
Conclusion: Infections acquired in burn centres are frequent and most of the patients experience several episodes. Burn wound and lungs mostly affected and micro-organisms have not infrequently a multi-resistant phenotype.

P631 Are *Staphylococcus epidermidis* infections nosocomial infections?

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Objective: *Staphylococcus epidermidis* strains causing catheter-associated infections display a high prevalence of the icaADBC gene cluster required for PIA-dependent biofilm formation as well as the mecA gene mediating methicillin resistance. In contrast, commensal isolates from healthy individuals display low prevalences for icaADBC and mecA suggesting a possible transmission of icaADBC and mecA-positive strains in hospitals.
Methods: We investigated the prevalence icaADBC and mecA in *S. epidermidis* strains from hospital environments (H-SE, n = 164) and non-hospital public environments (P-SE, n = 130). The clonal relation of representative icaADBC and/or mecA positive isolates was determined by MLST using the eBURST algorithm for analysis.
Results: Similar prevalence of icaADBC was observed in H-SE (20.7%) and P-SE (20.0%). In H-SE a significant higher prevalence of mecA (17.7%) was observed compared to P-SE (6.2%). Thereby, 13 of 34 icaADBC-positive H-SE were also positive for mecA (38.2%), whereas only 3 of 26 icaADBC-positive P-SE were mecA-positive (11.5%), indicating that the observed linkage of icaADBC and mecA in infection associated *S. epidermidis* is characteristic for hospital associated strains. Analysis of the clonal relation revealed also significant differences. 32 of 50 H-SE clustered in one clonal complex with MLST ST6 as primary founder and STs ST2 and ST5 as predominant clones, corresponding to the epidemiology evaluated in different studies for *S. epidermidis* isolates causing foreign body-associated infections. In contrast, only 10 of 30 P-SE were associated with this cluster and 18 isolated displayed MLST STs characterised as singletons. Interestingly, 6 H-SE with ST2 were mecA-positive, whereas the only P-SE with ST2 was icaADBC-positive and mecA-negative. All 7 H-SE of ST5 were icaADBC-negative and mecA-positive. In contrast, one of two P-SE with ST5 displayed a icaADBC-positive and mecA-negative genotype.
Results: Summarising the epidemiological data as well as the clonal relation of the investigated isolates it could be suggested that hospital specific environmental *S. epidermidis* clones exist which might be a source for transmission to patients with subsequent infection. Thereby, the linkage of icaADBC and mecA could result in the selection of biofilm-positive strains by antibiotic treatment.

P632 Results of the first national prevalence study on hospital-acquired infections in the Netherlands

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Objective: A national prevalence survey was set up to acquire national estimates of the prevalence of hospital-acquired infections (HAI), to offer leads for further research or interventions and to improve benchmarking possibilities.
Methods: In March 2007 the first Dutch national point prevalence study on HAI was organised. This study, set up by the PREZIES-network (network for "prevention of nosocomial infections through surveillance") and other working parties is repeated in March and October of each year. Infections are recorded according to a uniform protocol, using the CDC criteria with minor adaptations.
Results: In March 2007 30 hospitals participated, in October 2007 12 and in March 2008 29: in total 37 different institutions of the total of 96 Dutch hospitals.
The prevalence of HAI in 21,370 patients was 7.2% (95% confidence interval (CI) 6.9–7.6%). Of the patients 6.3% was infected. Table 1 shows the prevalence of all and the major infection types. Surgical site and symptomatic urinary tract infections occurred most frequently.

	Mean % (range)		
	All patients	non-ICU	ICU
SSI (n = 7712)	4.8 (1.9–14.3)	4.5 (1.5–14.3)	8.1 (0.0–60.0)
Pneumonia	1.1 (0.0–3.4)	0.6 (0.0–3.0)	9.5 (0.0–36.4)
Primary sepsis	0.5 (0.0–2.5)	0.4 (0.0–2.0)	2.4 (0.0–21.4)
Symptomatic urinary tract infection	1.7 (0.0–6.5)	1.7 (0.0–6.9)	2.1 (0.0–18.2)
All infection types	7.2 (2.2–15.4)	6.3 (1.5–15.3)	25.1 (0.0–100)

Of the patients with a urinary catheter on the survey day 3.6% (range 0.0–13.3%) had a symptomatic urinary tract infection. The percentage ventilated patients with pneumonia was 14.9% (range 0.0–100.0%). Secondary and primary sepsis, gastro-intestinal and skin infections occurred in 0.3–0.5% of the patients.
The use of urinary catheters on the survey day varied between 13.2 and 30.7%. Antibiotic use ranged from 22.0 to 42.9%. Of the patients with HAI 71.9% received antibiotics at the survey day and of those without 28.7%. At admission 3.6% of the patients had a HAI, 65.5% of which were acquired in the same hospital.
Multivariate regression, accounting for clustering per hospital (Proc Genmod in SAS) showed an increased risk for age (Odds ratio 1.1 (CI 1.0–1.1) per 10 years increase), specialism (anaesthesiology, surgery (not plastic surgery), traumatology, haematology compared to cardiology, dermatology, ENT, paediatrics, obstetrics & gynaecology and ophthalmology 1.7 (1.4–2.1)), surgery 1.3 (1.1–1.4), central venous catheter 1.8 (1.4–2.2), peripheral catheter 1.4 (1.3–1.6), invasive ventilation 1.7 (1.3–2.3), urinary catheter 1.7 (1.5–2.0) and length

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

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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 sentinels 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 medicals 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 schedule and a charter for a better national establishment. The setting in of this network is a real experiment to be shared with the other countries of our sub-region in order to federate a West African network.

P634 Seven years of SARI (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units; 2001–2007

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Objectives: To analyse data of SARI (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units from 2001–2007 and to look for temporal changes.
Methods: Prospective unit and laboratory based surveillance in 49 German ICUs from 2001–2007. The data were calculated on proportions of non-duplicate resistant isolates (RP), resistance densities (RD; i.e. the number of resistant isolates of a species per 1000 patient days) and antimicrobial usage density (AD) expressed as daily defined doses (DDD) and normalised per 1000 patient-days (pd). Linear trends were calculated using linear regression analysis with monthly data.
Results: Total mean antibiotic use (without sulbactam) stayed stable over time. AD was 1180 in 2001 and 1236 in 2007. Carbapenem use almost doubled to an AD of 134 in 2007. Significant increased were also calculated for quinolones (AD of 168 in 2007) and 3rd and 4th generation cephalosporin (3/4GC) use (AD of 124 in 2007). The most prominent decrease faced aminoglycoside use (AD was 86 in 2001 and 29 in 2007).
RRs were as follows in 2001 and 2007: MRSA 26 and 21%, VRE 0.6 and 1.7%, 3GC resistant *E. coli* 1.2 and 11.2% and 3GC resistant *K. pneumoniae* 3.8 and 10.5%.
The burden of resistance or the RDs did not change for MRSA but increased significantly for VRE and 3GC resistant *E. coli* and *K. pneumoniae*. RD of MRSA was 4.5 in 2007, of VRE 0.3 and of 3GC resistant *E. coli* and *K. pneumoniae* 2.2 resistant pathogens/1000 pd.
Conclusion: Total antibiotic use did not change over time with 1.2 DDD per patient per ICU day. However, there was a significant increase in the use of broad spectrum antibiotics like carbapenems. The burden of MRSA did not change in contrast to an increase in the burden of 3GCR *E. coli* and *K. pneumoniae* in German ICUs. In the face of resistant pathogens we find it better to describe and compare the burden of resistance and the magnitude of the public health problem by resistance densities besides resistance proportions.

P635 Inadequate drugs for the treatment of infections with Gram-positive pathogens using the EPICENTER Network data

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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 the drug–bug combinations in question is similar. Enterococi and Staphylococci show different susceptibility profiles. Only teicoplanin, vancomycin, rifampicin, and fosfomycin are active on all tested Gram positive pathogens, but the last two mentioned drugs are only for use in combinations. Aminoglycosides are active only on *S. aureus*. Surprisingly, even at high concentrations activity of aminoglycosides is low for the Enterococci. All quinolones seem to be of little value, even Moxifloxacin shows acceptable results only with *S. aureus*.
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.

Antimicrobial	Antibiotic resistance of Gram-positive isolates			
	E. faecalis	*E. faecium*	S. *aureus*	*S. epidermidis*
Gentamicin	**100.0%**	**100.0%**	5.00%	**45.40%**
Gentamicin-Syn	33.10%	**43.90%**		
Streptomycin-Syn	**44.00%**	**77.20%**		
Amoxicillin/Clavulanic acid	0.90%	**81.30%**	**32%**	**62.80%**
Ampicillin	0.90%	**87.10%**	**79.20%**	
Cefazolin			**32%**	**62.20%**
Cefoxitin			32.00%	**73.00%**
Imipenem	0.60%	**95.20%**	32%	**84.70%**
Meropenem	7.70%	**91.70%**	32.10%	**73.40%**
Oxacillin	**100.00%**	**100.00%**	32.00%	**73.40%**
Penicillin G	0.00%	0.00%	**76.40%**	**85.10%**
Trimeth/Sulfa	**100.00%**	**100.00%**	1.60%	33.60%
Fosfomycin w/G6P	0.40%	4.10%	0.80%	24.30%
Fusidic acid			1.40%	21.00%
Teicoplanin	0.10%	3.30%	0%	0%
Vancomycin	0.20%	3.30%	0%	0%
Clindamycin	**98.70%**	**95.90%**	24.10%	**49.40%**
Erythromycin	**50.50%**	**90.40%**	30.90%	**71.80%**
Mupirocin high level			1.40%	4.80%
Ciprofloxacin	**45.40%**	**86.30%**	**36.20%**	**61.20%**
Levofloxacin	**39.70%**	**84.60%**	**35.70%**	**55.00%**
Moxifloxacin	**36.00%**	**85.20%**	30.80%	**36.50%**
Ofloxacin			**45.10%**	**51.10%**
Rifampin			1.60%	8.10%
Tetracycline	**75.10%**	31.00%	4.80%	12.60%

P636 Ten-year trend in aminoglycoside resistance from a worldwide collection of Gram-negative pathogens (1998–2007)

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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 (listed 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: 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

Organism	% GEN R 1998–2000 (27,491 isolates)/2005–2007 (30,430 isolates)			
	NA	LA	EU	APAC
EC	3.2/9.3	10.4/15.4	6.4/7.8	15.5/43.1
KBS	3.9/7.9	30.5/38.4	20.9/12.3	12.0/37.6
EBS	5.8/8.7	21.2/29.9	9.7/10.1	12.8/30.4
CBS	3.2/3.8	26.5/12.5	14.1/5.0	5.4/28.4
SER	2.7/3.9	26.0/20.6	14.8/8.9	22.8/13.2
PM	6.4/6.3	36.1/28.1	20.2/11.0	6.2/21.5
IPP	11.5/12.4	20.5/43.9	15.9/12.0	21.5/24.3
PSA	14.7/12.6	38.6/36.7	30.0/22.6	15.7/33.6
ACB	31.6/42.7	65.6/67.9	69.2/63.0	35.3/60.0

[a]Isolates were non-S to GEN.

P637 *Klebsiella pneumoniae* 2002–2007: multi-class resistance and changing patterns of resistance to carbapenems and third-generation cephalosporins

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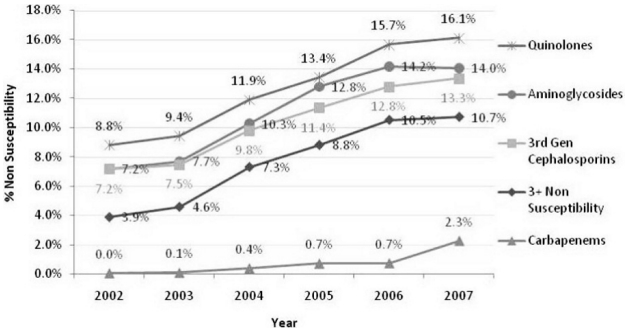
Objectives: To describe the changes over time of *K. pneumoniae* resistance to several antimicrobial classes in a statistical model that adequately adjusts for changes over time and the effect of several risk factors.

Methods: The surveillance study consisted of 186,819 US isolates from the TSN Network® surveillance database (Eurofins Medinet) for the period 2002–2007. Covariates available for analysis were time, age, sex, state, location (Ward or ICU) and source (blood, sputum, etc). Antimicrobial classes considered in the model were carbapenems, 3rd generation cephalosporins, aminoglycosides and fluoroquinolones. Multi-class or multiple resistance was defined as non susceptibility to three or more antimicrobial classes. Odds of resistance and 95% confidence intervals around rates were obtained using a logistic regression model.



Results: Non susceptibility to carbapenems was not observed in this data set in 2002 but reached 3% for Meropenem in 2007. 3rd Generation cephalosporins such as Ceftazidime showed a continuous increase from 8.9% in 2002 to 14.2% in 2007, with 70% of the latter being multiply resistant compared to 50% in 2002 ($p < 0.001$). Levofloxacin resistance had a similar increase from 7.6% in 2002 to 14.5% in 2007, with 69% of the latter also being multiply resistant. Although there is a tendency toward a plateau for aminoglycosides, such as Gentamicin and Amikacin, multiple resistance has increased 2.9 fold (2.7–3.2) in five years of surveillance from 3.9% in 2002 to 10.7% in 2007. A multivariate model for multiple resistance has it increasing 2.1%

(0.5–3.7%) every year (p = 0.001), with a 2.4% (0.8–3.4%) significant difference between ICU and Ward independent of time. Subjects under 18 years had a significantly lower rate (7.8%, 7.4–8.2%) in 2007 than those over 65 years old (11.2%, 10.9–11.6%).
Conclusion: *K. pneumoniae* non susceptibility to several antimicrobial classes has shown a marked increase during the surveillance period. Resistance to three or more antimicrobial classes has been amplified despite relatively low levels of carbapenem resistance and a plateau in aminoglycoside resistance.

P638 Susceptibility of baseline *Pseudomonas aeruginosa* and *Acinetobacter baumannii* to doripenem and other antibiotics from six doripenem phase 3 clinical trials

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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 IMI. When DOR MIC was 4 mg/L (n = 12), 25% of *P. aeruginosa* had IMI MIC $\leqslant$ 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).

P639 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.

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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) and 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.

Organisms	Hospitals			Commercial Lab		
	2003	2005	2007	2003	2005	2007
Oxacillin-R *S. aureus*	68	59	64	55	53	63
Oxacillin-R CoNS	73	70	64	69	53	70
Vancomycin-R *E. faecalis*	1	2	1	0	0	1
Vancomycin-R *E. faecium*	20	20	21	7	12	17
Cefotaxime-R *E. coli*	11	12	11	12	12	20
Cefotaxime-R *K. pneumoniae*	20	25	25	18	39	45
Cefoxitin-R *E. coli*	10	8	6	6	8	11
Cefoxitin-R *K. pneumoniae*	23	24	21	18	33	35
Imipenem-R *P. aeruginosa*	20	19	21	17	19	29
Imipenem-R *Acinetobacter* spp.	13	16	22	5	9	20

P640 Susceptibility to levofloxacin-centred fluoroquinolones and other antibiotics of 19 species, 12,919 clinical isolates collected from 72 centres in Japan, 2007

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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 cephems 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 FQs-resistant rate in methicillin-resistant *Staphylococcus aureus* (MRSA) was approximately 90% except for sitafloxacin while FQs-resistance rate in methicillin-susceptibl *S. aureus* was around 5%. In *Pseudomonas aeruginosa* clinical isolates, 25–30%

from UTI and 10–20% of from RTI were resistant to FQs. Carbapenem-resistant *Acinetobacter* spp. 16 isolates (*A. baumannii* 14, *A. lwoffii* 1, *A. haemolyticus* 1) were found in 598 isolates and these resistant isolates were focused in some particular facility.
Conclusion: It was confirmed that FQs including Levofloxacin have still strong activity against the common respiratory pathogens and Enterobacteriaceae except for *E. coli*. Increase of macrolide resistance was remarkable in A group streptococci. Outbreak of carbapenem-resistant *A. baumannii* began to show a sign in Japan.

P641 Molecular characterisation of fluoroquinolone resistance in *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae* and *Escherichia coli* clinical isolates collected from 72 centres in Japan, 2007

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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: QRDR 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 possesed 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 ⩾4 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 numbers of mutation point in QRDRs. 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.

P642 Antimicrobial resistance and in vitro biofilm-forming ability of *Pseudomonas aeruginosa* isolates from veterinary origin

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Objectives: *Pseudomonas aeruginosa* is an important human opportunistic pathogen, but is also relevant for animals. Treatment options are declining, due to massive antibiotic use. Antimicrobial resistance mechanisms include intrinsic low cell wall permeability, acquired beta-lactamases and aminoglycoside-modifying enzymes and efflux pumps, and may be further enhanced by biofilm production. We aimed to investigate the relation between antimicrobial resistance and biofilm formation in *P. aeruginosa* from veterinary origin.
Methods: Antimicrobial resistance of *P. aeruginosa* animal isolates from house pets, farm and zoo animals (n = 34), with clinical signs of infection, previously characterised by fAFLP 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, cephalexin, cephotaxim, nalidixic acid, penicillin G, tetracycline, 100%; chloramphenicol, 97.96%; sulphametoxazole/trimethroprim, 97.06%; streptomycin, 88.24%; enrofloxacin (ENR), 61.76%; carbenicillin (CAR), 67.65%; gentamicin (GEN), 47.06%; cephoperazone (CFP), 44.12%; amikacin, 32.35%; piperacillin, 29.41%; ofloxacin, 23.53%; ciprofloxacin, 17.65%; ceftazidime, 11.76%; imipinem, tobramicin, 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.

P643 Surveying aminoglycoside resistance mechanisms: a tool for the development of neoglycosides

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Objectives: To support the development of neoglycosides, the next generation of aminoglycosides (AG) 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 AGs in the test population matched those in the entire collection. MICs of 9 AGs 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 multidrug resistant (MDR) 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 AGs 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 with the 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/efflux and now due to ribosomal methyltransferases (RMT). In all 3 studies the

kanamycin-/neomycin-resistance phenotype caused by APH(3′)-I/II was not evaluated.

Conclusions: These results demonstrate that while the overall prevalence may be increasing (companion abstract by Biedenbach et al), the distribution of AGRM amongst AG-resistant isolates worldwide remained stable during the past 20 years. New agents that evade these AGRM, such as neoglycosides, are needed.

AGRM	Phenotype	Percentage of AG-R isolates with given AGRM[a]					
		CID 1988–1993 n = 10,181		SENTRY 2005 n = 406		SENTRY 2007 n = 302	
		Alone	Comb.	Alone	Comb.	Alone	Comb.
AAC(3)-I	G	1.2	8.3	0.7	12.1	0.3	6.3
AAC(3)-II	GTN	20.9	17.5	12.3	23.7	23.2	15.2
AAC(3)-IV	GTN	1.7	0	0.7	2.0	0.3	1.0
AAC(2′)-I	GTN	2.1	1.1	0	0	0	0
AAC(6′)-I	TNA	10.4	21.8	8.6	25.9	9.6	19.2
AAC(6′)-II	GT	3.6	3.0	7.4	2.7	1.7	4.0
ANT(2″)-I	GT	7.2	4.3	15.0	7.4	2.7	4.6
APH(3′)-VI	A	0.1	3.4	1.0	4.7	1.0	9.3
Perm/Efflux	GTNA	7.6	7.4	2.0	6.4	12.9	11.3
RMT	GTNA	0	0	5.2	0.2	15.6	ND[b]
	Total	54.8	66.8	52.9	85.1	67.3	70.9

[a]Comb. = AGRM present in combination with one other or more AGRM.
[b]ND = No data.

P644 Antibiotic susceptibility of *Bacteroides* clinical isolates in Europe, 2007–2008

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Objective: *Bacteroides* strains are the most frequently isolated anaerobic bacteria causing serious infections alone or in mixed cultures. Due to several special resistance mechanisms antibiotic resistance occurs among these bacteria, which may lead to treatment failure using empiric antibiotic therapy. The aim of this study was to evaluate the antibiotic susceptibilities of recent clinical isolates of *Bacteroides* strains collected from different European countries and to follow changes in this respect in Europe.

Methods: Consecutive, recent clinical isolates were collected from serious infections. Altogether 824 isolates from 13 countries were involved in this study belonging to different species of *Bacteroides*. Antibiotic susceptibilities were measured by agar-dilution method using Brucella agar (BBL Microbiology Systems, USA) supplemented with 5 mg haemin and 1 mg vitamin K per ml and 5% leaked sheep blood. MIC values of ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefoxitin, imipenem, clindamycin, metronidazole, moxifloxacin and tigecyclin were measured. Overall resistance rates and frequency of multi-resistant isolates were evaluated. EUCAST breakpoints were used for all antibiotics where available.

Results: High level resistance rates were found for ampicillin, only <0.7% of the isolates had an MIC lower than the resistance breakpoint. Amoxicillin-clavulanic acid as well as piperacillin-tazobactam were active against 89.6% and 89.9% of the isolates, respectively. Most of the isolates (84.6%) had an MIC > 4 mg/l for cefoxitin and clindamycin resistance was quite frequent in some countries. Altogether 31.3% of the isolates had a clindamycin MIC higher than the resistance breakpoint. Imipenem, metronidazole and tigecyclin were the most active anti-anaerobic drugs against *Bacteroides* strains in this study. Only 0.8%, 0.2% (according to the EUCAST breakpoint) and 1.7% (according to the CLSI breakpoint) of the strains were resistant, respectively. Moxifloxacin activity was influenced by the country where the strains were isolates. The overall resistance to moxifloxacin was 15% according to the CLSI breakpoint.

Conclusion: As in many countries resistance determination of anaerobic bacteria is not always carried out in the routine laboratories, it is mandatory to follow the resistance status of these important pathogens on the European level in the future as well.

P645 Molecular analysis of recent cephamycin and carbapenem-resistant *Bacteroides* isolates

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Objectives: Our aim was to investigate 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. Comparision of the 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 ISBf8 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 resitant (MIC ⩾ 16 mg/l) of wich 5 were cfiA-positive. Among these latter strains 4 harboured IS elements (2 IS614-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 cfxA gene among *Bacteroides* strains and probably to their cefoxitin heteroresistant phenotype.

P646 Antimicrobial susceptibility of food-borne bacteria (*Salmonella*, *Campylobacter*) from cattle, pigs and chickens (2002–2004) recovered from 8 EU countries (EASSA programme)

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Objective: Nontyphoidal *Salmonella* and *Campylobacter* are a major cause of food-borne illness. For severe illness, fluoroquinolones and 3rd generation cephalosporins for salmonellosis; and macrolides for campylobacteriosis are the treatment of first choice in the EU. Antimicrobial susceptibility to human-use antibiotics was investigated among *Salmonella* spp., and *C. jejuni* and *C. coli* from healthy cattle (Ca); pigs (P); and chickens (Ch) at slaughter across the EU.

Methods: Colon or caecal content was randomly collected at 4 abattoirs/country (n = 5 per host). Each herd/flock was sampled once. *Salmonella* and *Campylobacter* were isolated using standard methods. Susceptibility testing was done by agar dilution (CLSI, M31-A2) in a central laboratory. Clinical resistance per drug/organism/country was based on the recommendations of CLSI (M45-A; M100-S17); decreased susceptibility (DS) was based on epidemiological cut-off values as defined by EFSA (2007).

Results: In total 406 *Salmonella* (Ca, n = 24; P, n = 271; Ch, n = 111) comprising 37 serovars were recovered. Clinical resistance to

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.

P647 Antimicrobial susceptibility of commensal bacteria (*Escherichia coli*, *Enterococcus*) from cattle, pigs and chickens (2002–2004) recovered from 8 EU countries (EASSA programme)

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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, 0; gentamicin (G) 1.8, 1.5, 3.4; tetracycline 13, 68, 67; and 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, Ch), 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. faecalis* 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.

P648 Antibiotic susceptibility of invasive *Neisseria meningitidis* isolates from 1995 to 2008 in Sweden – the meningococcal population remains susceptible

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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, penicillin V, cefotaxime, chloramphenicol, ciprofloxacin, rifampicin, and sulfadiazine were determined using the Etest method on Mueller-Hinton agar supplemented with 5% heated ("chocolated") horse blood. All isolates were also tested for beta-lactamase production.

Results: All isolates were fully susceptible to cefotaxime (MIC $\leqslant$ 0.12 mg/l) and ciprofloxacin (MIC $\leqslant$ 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 $\leqslant$ 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

P649 The immunogenicity and protective immunity for a novel genetic vaccine against *Mycobacterium tuberculosis* with recombinant Plasmid expressing antigen Ag85A fused to cytokine

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Objective: BCG provides dissatisfactory protection against tuberculosis. Although DNA vaccines encoding Ag85A can induce strong immune response in vivo, the relatively low protective immune efficacy 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 to *Mycobacterium tuberculosis*. 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 recombinant 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.

P650 IgA levels in lung and serum after oral immunisation with BCG encapsulated in alginate microspheres

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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 CL7/L12 recombinant fusion protein and lipopolysaccharide

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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 HSA-L7/L12 fusion protein with LPS in Balb/c mouse.
Methods: The amplified L7/L12 gene was cloned in pYHSA5 vector then pYHSA5-L7/L12 construct was transformed in *Saccharomyces cerevisiae* and expressed protein from supernatant was purified by affinity chromatography column. LPS was extracted by n-butanol, purified by ultracentrifugation. Balb/c mouses were immunised in 9 groups with PBS, HSA, tHSA_L7/L12, L7/L12, LPS, LPS+HSA, LPS+tHSA_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 tHSA-L7/L12 exhibited a dominance of immunoglobulin IgG1 over G2a (IgG2a). LPS-HSA 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 tHSA-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 ($p \leqslant 0.005$).

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

Vaccine	Log CFU/spleen, mean±SD	Log protection	*p* value
PBS	4.96±0.23	0.00	>0.05
HSA	4.86±0.26	0.1	>0.05
L7/L12	3.75±0.27	1.2	<0.05
HSA-LPS	3.91±0.16	1.0	<0.05
LPS	4.271±0.02	0.7	<0.05
HSA-L7/12+LPS	3.01±0.14	1.9	<0.05
L7/L12+LPS	3.45±0.15	1.5	<0.05
S19	2.14±0.18	2.82	<0.01

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

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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 10 mM tris buffer, and 1 mg each of DNase and was added per 100 ml. 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-lauroyl sarcosinate. Resultant insoluble material was dialyzed against tris buffer. In order to isolate the peptidoglycan from outer membrane proteins, lysozyme (1 mg/50 mg of membrane protein) was added. Samples were finally ultracentrifuged and supernatants were kept at 4ºC. 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 *B. abortus* 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.

Results: Among the three different compounds applied to promote humoral immune responses in the animal models, *B. abortus* Porins in combination with *B. abortus* S99 LPS have been the most potent immunogen that significantly elicited the highest IgG titer against *B. abortus* ($P < 0.05$). The difference between IgG titer following the immunisation with *B. abortus* Porins and *B. abortus* Porins + Complete Freund's adjuvant has not been significant ($P > 0.05$).
Conclusion: The application of both *B. abortus* Porins and *B. abortus* LPS would be a promising aspect to design a human brucellosis subunit vaccine since Porins are able to promote T-dependent and Cell mediated responses while the highest titers of anti-*Brucella* IgG antibodies are directed against LPS.

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

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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 –20ºC. Presence of anti-Brucella antibodiesin the sera of immunised animals demonstrated by Rose Bengal test (RBT), Serum agglutination test (tube agglutination and rapid slide agglutination) and Agarose Gel Immunodiffusion (AGID).
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 titers 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*

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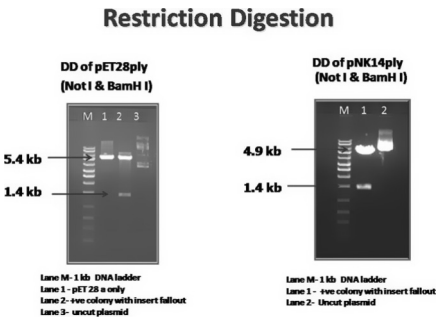
Objectives:
a. PCR amplification of wild Ply gene (1432 bp);
b. Cloning of wild ply gene into pGEM-T Easy vector;
c. Site directed mutagenesis of wild ply-pGEM-T construct;
d. Construction of a pneumolysin DNA vaccine using pNK14 vector.
Methods: Wild type ply gene was PCR amplified and subjected to TA cloning using pGEM-T Easy Vector (3015 bp) and construct after sequence confirmation was subjected to deletion at Ä146AA 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, Pst1, & Apo1) and double enzyme restriction digestions (BamH1 & Acc65I) and final confirmation by sequencing.
c. Site directed mutagenesis (Ä146AA 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 parental DNA template and final confirmation of deletion is confirmed by mutant gene sequencing.
d. Sub-Cloning of MPly in pNK14 Vector: Mply-pGEMT and pNK14 vector was double digested mixed and ligated using T4 DNA ligase. The recombinant clones were confirmed by double digestion and sequencing. Similarly Mply-pGEMT was sub-cloned in pET28a vector. Double Digestion of pET28ply and pNK14ply using Not I and BamH I is shown in graphics.



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-tPA pNK14 DNA is based on ply gene fused with a signal peptide sequence tPA (tPA-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

P655 First results of the new Dutch Infectious Diseases Surveillance Information System-Antimicrobial Resistance (ISIS-AR). Epidemiology of extended-spectrum beta-lactamases in the Netherlands

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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 (I/R) to 3 d 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 practioners (GP).

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

Results: The prevalence of blood isolates I/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 I/R 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 to 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 positive EC or KP in the community is compromised, (4) other resistance mechanisms than the classical ESBLs probably play a role in the decreased susceptibly to CEPH3 as well.

P656 High prevalence of extended-spectrum β-lactamase production in *Enterobacter* spp. bloodculture isolates in the Netherlands

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Objective: In 2008 a guideline of the Dutch Society for Medical Microbiology for screening and confirmation of ESBLs in Enterobacteriaceae was issued. An optional recommendation in the guideline was to screen *Enterobacter* spp. for ESBLs (i.e. MIC > 1 mg/L for cefotaxime and/or ceftazidime) and to perform a confirmation test with cefepime ± clavulanic acid. Dutch microbiologists questioned this recommendation because no data were available indicating that *Enterobacter* spp. might be an important source for ESBLs.

The aim of this study was to determine the prevalence of ESBLs in *Enterobacter* spp. causing invasive infections and to obtain their susceptibility patterns.

Methods: *Enterobacter cloacae* and *Enterobacter* Aerogenes bloodculture isolates obtained in 2006 and 2007 in 12 hospitals throughout The Netherlands were collected (one isolate per patient). Susceptibly testing for 22 antimicrobial agents using broth microdilution (18) or Etest (4) was performed on all isolates as well as the ESBL confirmation Etest with cefepime/cefepime + clavulanic acid. EUCAST breakpoints were used.

Results: 272 isolates were obtained (83.5% *E. cloacae*). Of the total collection of 272 isolates 41.5% (113) was positive in the screentest of which 30% was positive in the confirmation test, reflecting an ESBL prevalence of 12.9% (35/272), ranging from 0% to 33% per hospital. *E. cloacae* isolates were ESBL positive in 14.5% and E. Aerogenes in 4.4%. The confirmation test was indeterminate in 2.9% (8/272) of the isolates. Molecular analysis of ESBL genes is pending.

Susceptibilty rates in ESBL positive versus ESBL negative isolates were as follows: for meropenem 97% vs 97%, respectively; for imipenem 91% vs 95% (NS); for ertapenem 43% vs 86% ($p < 0.001$); for cotrimoxazole 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).

Conclusions: 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 ertapenem, ciprofloxacin, tobramycin, amikacin and cotrimoxazole.

P657 Acquisition of cephalosporin-resistant Enterobacteriaceae in relation to global exposure to antibiotic β-lactams in ten intensive care units of Paris metropolitan area

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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 beta-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 "naive" 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 ceftazidime (diameter $\leqslant$17 mm, according to Clinical and Laboratory Standards Institute 2008) or to cefotaxime (diameter $\leqslant$22 mm), or producing extended-spectrum β-lactamase (Etest ESBL strip containing cefepime-clavulanate). 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 naive 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 naive 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.

P658 Cross-contamination does not explain the increase in ESBL-producing isolates in intensive care units in a French university hospital

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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, of the 72 ESBL-producing isolates from 57 patients (41 colonised and 16 infected), 27 (37.5%) were *E. coli*, 21 (29%) *K. pneumoniae*, 17 (23.5%) *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 isolates detected after 48 h 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.

P659 Factors associated with colonisation with extended-spectrum beta-lactamase-producing enterobacteria in newborns hospitalised at the neonatal intensive care unit

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Objectives: Colonisation and infection with extended-spectrum beta-lactamase producing enterobacteriaceae (ESBL-E.) are a growing problem in Neonatal Intensive Care Units (NICU). Preterm low birthweight, prolonged mechanical ventilation, invasive devices and prior use of third generation cephalosporines were reported to be risk factors for colonisation and/or infection with ESBL-E. In the last years several outbreaks of ESBL-E. colonisation occurred at our NICU.
Methods: We retrospectively analyzed risk factors associated with colonisation with ESBL-E. in newborns hospitalised at the NICU from Jan. 2005 to Jul. 2008. Patients were screened routinely at least twice a week for ESBL-E. in stool.
Fisher's exact test and Mann-Whitney-U test (SPSS for Windows) were used for statistical analysis.
Results: 69 (5.9%) out of 1164 patients have been colonised with ESBL producing *Klebsiella pneumoniae* (n = 51), *Klebsiella oxytoca* (n = 11), *Serratia marcescens* (n = 6) and *E. coli* (n = 1). For details see Table 1.
Cefuroxime/ampicillin were used as first line antibiotic regimen. Third generation cephalosporines were not used during the observation period.
Conclusion: ESBL gene expression might be induced under therapy with second generation cephalosporines.
Factors associated with more severe illness (including lower gestational age and birth weight and more invasive therapeutic procedures) leading to longer stay at the NICU were significantly associated with colonisation with ESBL-E. Longer hospital stay increases the risk of patient-to-patient transmission.

Table 1

	ESBL non-colonised patients n = 1095 (94.1%)	ESBL colonised patients n = 69 (5.9%)	p-value p-value
	number (%)		*Fisher's exact test*
Female gender	506 (46.2)	36 (52.2)	n.s.
Cesarean section	712 (65.0)	55(79.7)	0.013
Apgar 1 <9	563 (51.4)	57 (82.6)	<0.001
Apgar 5 <10	555 (50.7)	54 (78.3)	<0.001
Apgar 10 <10	427 (39.0)	49 (71.0)	<0.001
Respirator therapy	366 (33.4)	44 (63.8)	<0.001
CPAP therapy	353 (32.2)	37 (53.6)	0.001
CVC	70 (6.4)	12 (17.4)	0.002
	median (range)		*Mann-Whitney-U*
Gestational age	35 (23–43) weeks	31 (24–40) weeks	<0.001
Birth weight	2365 (400–5215) g	1415 (540–3834) g	<0.001
Respirator days	0 (0–66)	3 (0–36)	<0.001
CPAP days	0 (0–63)	1 (0–86)	<0.001
CVC days	0 (0–37)	0 (0–28)	<0.001
Stay	15 (1–198) days	48 (3–194) days	<0.001
Clean stay	15 (1–198) days	22 (1–194) days	0.005

CVC: central venous catheter; Clean stay: stay before/without ESBL colonisation.

P660 Bacteraemia by extended-spectrum beta-lactamase-producing *Escherichia coli* as a complication after endoscopic retrograde cholangiopancreatography

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Background: The present was performed to describe the characteristics of bacteraemias by Extended-spectrum beta-lactamase-producing *Escherichia coli* following cholangiopancreatography and the methods of prevention and treatment of the same.
Methods: Thirteen different episodes of bacteraemia by Extended-spectrum beta-lactamase-producing *Escherichia coli* were retrospectively studied in patients submitted to endoscopic cholangiopancreatography (ERCP) from 2006–2008. All the patients received antimicrobial prophylaxis with piperacillin-tazobactan (4.5 gr/8 hours during 24 hours).
Results: Thirteen patients (3.6% of the patients undergoing ERCP) presented episodes of bacteraemias by the microorganism in the 48 hours following the procedure. 53.6% were male, mean age 70 years, limits 44–85). A biliar prosthesis was put during the procedure in eight patients. All the patients presented obstructive disease in the form of calculi or neoplasms at the level of the biliary tree. All the patients showed typical clinical manifestations of cholangitis. One patient (7.7%) died as a consequence of bacteraemia and a hepatic abscess was developed in other case. The prosthesis was removal on trhee cases.
Conclusions: Bacteraemia by Extended-spectrum beta-lactamase-producing *Escherichia coli* following ERCP is more frequent in patients with obstructive disease of the biliary tract and has an important additional morbimortality. The use of drugs with an betalactamase-inhibitor as preendoscopic prophylaxis probably should be considered as predisposing factors.

P661 ESBL *E. coli* bacteraemia: clinical influence at a university-affiliated hospital

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Objectives: Incidence of ESBL *E. coli* bacteraemia (ESB) has progresively increased but its repercusion in mortality and mortality is not clearly known. The aim of this study was to evaluate epidemiological and clinical aspects in a cohort of patients with ESB.
Patients and Methods: Observational and comparative study of a cohort of non-paediatric patients with ESB 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 ESB 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. Mortality was assessed till 30 days after diagnosis of ESB.
Results: 150 patients with *E. coli* bacteraemia were studied; prevalence of ESB was 17% (n = 26; 12 female). Mean age was 57 years (range 14–94); 14 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. A predisposing factor was identified in 19 patients (73%): previous antibiotic treatment (9), previous hospitalitation (8), urinary catheter (8), surgical catheters (5) and post-surgical period (5). An underline condition was present in 22 patients (84.6%): cancer (11), obstructive uropathy (6), diabetes (5). Charlson comorbility 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. Origen of ESB 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 ESB is necessary to improve empirical treatmets and dicreased associated mortality and morbility.

P662 In vitro activity of beta-lactam antibiotics against CTX-M-producing *E. coli*

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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-amplicons were performed. MIC for amoxicillin-clavulanic acid, aztreonam, cefepime, cefotaxime, ceftazidime, ceftibuten, ertapenem, imipenem, mecillinam, meropenem, piperacillin-tazobactam and temocillin were determined using Etest. The susceptibility was determined according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing. MIC50 and MIC90 were calculated.
Results: One-hundred thirty-two isolates belonged to CTX-M group 1 and 55 isolates to CTX-M group 9. Susceptibility of *E. coli* from CTX-M group 1 and 9 are shown in the table. Isolates with CTX-M group 9 enzymes showed higher susceptibility to the beta-lactam antibiotics tested than isolates belonging to CTX-M group 1. More than 90% of the isolates belonging to CTX-M group 9 was susceptible for amoxicillin-clavulanic acid, ceftazidime, ceftibuten, piperacillin-tazobactam and temocillin. The isolates belonging to both CTX-M-groups showed high susceptibility (92.5%) to mecillinam. All isolates were susceptible to imipenem and meropenem and 98.4% to ertapenem.
Conclusions: This study show significant difference in susceptibility to different beta-lactam antibiotics among the CTX-M producing *E. coli* isolates and a significant difference for many antibiotics tested between the CTX-M producing groups 1 and 9. The good in vitro activity of other beta-lactam antibiotics than carbapenems against CTX-M-producing *E. coli* indicate that clinical studies are warranted to examine these beta-lactam antibiotics potential role in the treatment of infections caused by multiresistant ESBL-producing *E. coli*.

Antibiotic	MIC range (mg/l)	MIC_{50} (mg/l)	MIC_{90} (mg/l)	Susceptibility (%)
Amoxicillin-clavulanic acid	4–16	8	16	84.5
Aztreonam	0.5–>128	16	128	5.3
Cefepime	0.125–>128	4	32	5.3
Cefotaxime	2–>128	32	>128	0
Ceftazidime	0.125–>128	4	16	33.7
Ceftibuten	0.125–>128	4	16	34.8
Ertapenem	0.008–2	0.032	0.25	98.5
Imipenem	0.125–1	0.25	0.25	100
Meropenem	0.008–025	0.008–0.25	0.064	100
Mecillinam	0.125–>128	0.125–>128	8	92.5
Piperacillin-tazobactam	0.125–>128	0.125–>128	16	86.1
Temocillin	1–32	8	16	81.8

P663 In vitro activity of 13 antibiotics against CTX-M ESBL-positive and -negative clinically significant *Escherichia coli* isolates from 8 major hospitals in Kuwait

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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 consecutive 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 ciprofloxacin 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.

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

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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 [OR: 0.149, p = 0.007], ICU hospitalisation [OR = 0.23, p = 0.04], carriage of nasogastric tubes [OR: 0.29, p-0.035] and central venous catheters [OR: 0.266, 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 mechanical 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

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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), cefoxitin (FOX), ceftriaxone (CRO), 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 microg/mL. IMP-resistant isolates were examined by E-Test (AB Biodisk) for detection of metallo-beta-lactamases (MBL). In addition the genes encoding MBL (vim, 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 intermediately 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 intermediately 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

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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 (89.2–94.8%), piperacillin-tazobactam (89.0–93–9%), gentamicin (81.9–89.4%), ceftazidime (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%), ceftazidime (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 carbapanems 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 espectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in Colombia, 2002–2008

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Objective: To describe the trend in time of the frequency of *E. coli* and *K. pneumoniae* isolates with extended beta lactamase (ESBL) production in colombian thrid 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, Biomerieux). Data was systematised with Whonet 5.4®. Isolates were characterised according to ward type (ICU vs. non ICU) and sample type. Monthly

information (78 consecutive periods) was analysed with time series methodology (Box Jenkins) for each microorganism according to its localisation. With the best model, forecasts were realised for the next 12 months with confidence intervals of 95% by means of Statgraphics Centurión XV® software.

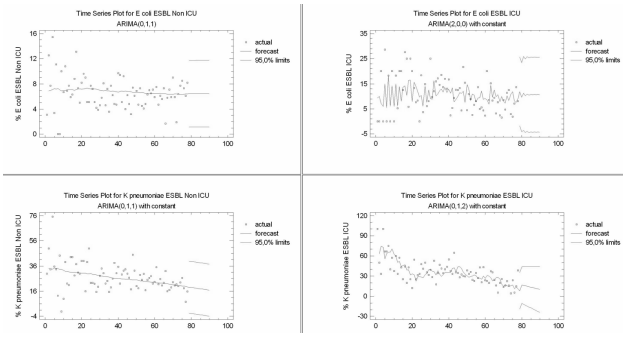

Figure 1. Time series and forecasting for ESBL-producing Enterobacteriaceae. GREBO 2002–2008.

Results: 14055 *E. coli* isolates and 4627 *K. pneumoniae* isolates were analysed. Global frequency of ESBL production was 6.9% (n = 976) for *E. coli* (samples were most frequently obtained from urine 48.5%, secretions 14.3%, blood 8.2%, faeces 6.9% and dialytic fluid 4.8%). ESBL were found in 27.81% (n = 1287) of *K. pneumoniae* isolates (obtained in 29% from urine, 22% from blood, 11.7% form secretions 8.4% from catheters and 7% from faeces). Annual frequency for ESBL in *E. coli* from the years 2002, 2003, 2004, 2005, 2006, 2007 and the first semester of 2008 were 8.1%, 9.1%, 6.7%, 7.1%, 6.1%, 6.3% and 7.4%, respectively. ESBL was found in *K. pneumoniae*, for the same years, with a frequency of 39.3%, 34.8%, 28.6%, 33.6%, 27.0%, 22.5% and 18.1%, respectively. Time series models and the forecast for the next 12 months is showed in the figure 1.
Conclusions: There is trend for a lower rate of ESBL isolates among *K. pneumoniae* in ICU and non ICU samples. The frequency of ESBL producers in *E. coli* has remained stable and there is slight increase for the last 18 months in non-ICU wards. The forecast for the latter is remain in a similar level.

P668 Dynamics of extended-spectrum β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*: a mathematical model

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Objectives: The prevalence of infections caused by extended-spectrum β-lactamases (ESBL) producing bacteria, associated with increased mortality, length of stay and costs, is rapidly increasing. Here we aim 1) to elucidate the dynamics of spread of ESBL and 2) to estimate the expected equilibrium prevalence of ESBL+ *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) in a single hospital and its catchment area.
Methods: We use a deterministic mathematical model with 3 hospital compartments distinguished by the risk for patients to acquire ESBL: Intensive Care Units (ICUs), high-risk non-ICU wards (e.g., haematology) and low-risk wards. Outside the hospital 3 patient groups are distinguished based upon their hospitalisation rates (data University Medical Center Utrecht (UMCU)). EC and KP can be ESBL + or –. Assuming each individual carries EC, we distinguish 6 intestinal colonisation states, EC–, EC–/KP–, EC–/KP+, EC+, EC+/KP–, EC+/KP+. Within the hospital, patients can acquire ESBL bacteria by cross transmission or by conjugation, when a patient is already colonised with a ESBL+ KP or EC. Within the hospital patients remain ESBL+ and after discharge lose ESBL+ strains after a mean of 90 days and do not acquire ESBLs. The nosocomial ESBL prevalence data used are deduced from the Dutch EARSS data (2000–2008) and UMCU (1996–2008) with a percentage of ESBL+ blood cultures in 2007 of 7% and 4% for KP and EC.
Results: Model simulation results fit well with observed prevalence rates in recent years. Inside and outside the hospital the prevalence of ESBL+ carriership 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

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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) or Etests (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 XbaI 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

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Objective: Infections due to extended-spectrum β-lactamase (ESBL)-producing by Gram-negative urinary pathogens in nonhospitalised

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 Clinico 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 ($P < 0.009$). 56% were from outpatients and 43% from emergency room patients. From 60 patients (32%) without a previous hospital admission 55 (39%) were diagnosed with UTI ocassionated 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% ($P = 0.27$), comorbility 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 59%. 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

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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, 7.2%; *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

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Background: Extended-spectrum β-lactamases (ESBL) 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*

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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 ceftazidime 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 do not seem to play a major role in their dissemination.

P674 Molecular fingerprinting of faecal isolates of *E. coli* with and without ESBL-phenotype in patients with intra-abdominal infections

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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 GTG-5-PCR method. Fingerprinting data and MICs were evaluated and isolates clustered using the bioinformatics software BioNumerics.
Results: Each patient was sampled 3–12 times during a period of 10–64 days. From each patient 2–17 isolates were detected. 43 of the 76 isolates had an ESBL-phenotype. Multi resistant isolates (i.e. resistant for 3 or more of β-lactam antibiotics, aminoglycosides, trimethoprim-sulfa and/or ciprofloxacin) were isolated from 2 patients. CTX-M genes belonging to group 1 were found in 3 patients and group 9 in 2 patients. SHV genes were found in one patient (SHV-1-like). Each patient's *E. coli* were divided in 1–7 fingerprinting patterns when subjected to semi-automatic GTG-5-PCR. No pattern of an isolate was 100% identical to any pattern from a different patient. In 2 patients the isolates with ESBL-phenotype differed completley from isolates without ESBL, but in the remaining 7 patients ESBL-phenotypes shared fingerprinting pattern with isolates without ESBL.
Conclusion: This study shows that it is possible with the GTG-5-PCR method to detect each patient's clones of *E. coli* since no patients had 100% identical clones. However it was not always possible to detect *E. coli* with ESBL genes among the other faecal isolates of *E. coli* with different antibiograms within the same patient. This indicate that most patients have several clonal related faecal isolates of *E. coli* with and without ESBL-genes and ESBL-phenotype with different antibiograms.

P675 Extended-spectrum beta-lactamase-producing Enterobacteriaceae: recent evolution and clinical impact in a large tertiary-care Italian hospital

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Objectives: The extended-spectrum beta-lactamases (ESBLs)-producing Enterobacteriaceae represent a major public-health concern. Epidemiology of ESBLs has recently undergone a remarkable evolution in several settings, mostly due to the massive diffusion of CTX-M-type enzymes vs. the older TEM- and SHV-type ESBLs. 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 ceftazidime 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

P676 Multiresistant urinary tract isolates of *Escherichia coli*: is it an issue in the empiric management of acute uncomplicated cystitis?

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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 >16 ys 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 $\leqslant 0.05$ 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,

CEP 6.7, FUR 4.6, COT 18.4, NAL 5.9, CIP 1.8, AMC 2, CXM 1.6, MEC 2.7 and FOS 0.8. Of these isolates, 6% were multiR (44/731). The predominant multiR phenotype included R to AMP-COT-NAL (12/44, 27.3%). Among multiR isolates, %R rates were: AMP 97.7, COT 77.3, CEP 63.6, NAL 61.4, FUR 31.8, CIP 22.7, CXM 22.7, AMC 20.5, MEC 9.5 and FOS 4.5. Use of fluoroquinolones in the previous 3 months (OR 3.66, 95%CI 1.82–7.37, $P < 0.001$) and age >65 ys (OR 2.22, 95%CI 1.46–3.39, $P < 0.001$) were significantly associated with multiR *E. coli* isolation in the multivariate analysis (model variables: UTI history, antibiotic use in the previous 3 months, hospital admission, diabetes mellitus, age).

Conclusions: Prevalence rates of *E. coli* urinary isolates multiR are significant and call for continuous surveillance, especially among elderly patients. For AUC empiric treatment, increased COT %R undermine its use as first-line agent. Although CIP exhibits low %R, caution in fluoroquinolone use as first-line for the empiric AUC treatment is prompted by: (a) increased NAL %R, (b) quinolone-resistant strains being frequently multiresistant, and (c) fluoroquinolone use association with multiR *E. coli* isolation. Mecillinam, fosfomycin and nitrofurantoin could serve as first-line choices, in the context of fluoroquinolone-sparing regimens.

P677 The effect of sewage treatment on antibiotic resistance of enterobacteria

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Objective: 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 in duplicate onto 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.

Resistance percentages of Enterobacteriaceae in raw and purified wastewater

	Percent resistant				
	Trimethoprim	Tetracycline	Ciprofloxacin	Nalidixic acid	Streptomycin
Influent	7.4	7.1	0.6	5.4	5.5
Effluent	11.5	15.2	0.7	7.5	8.8

P678 Surveillance of antibiotic resistance in urinary coliforms in Greater Lincolnshire, U.K. and the potential for temocillin, ertapenem and mecillinam usage

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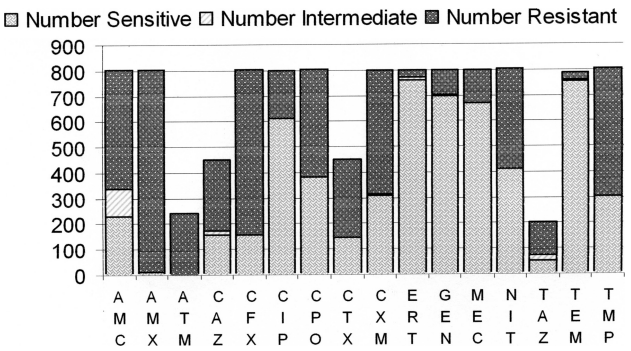
Objective: Surveillance of antibiotic sensitivity patterns in resistant urinary coliforms for a large, rural population over a recent six month period.

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 U.K. 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 clavulanate; AMX = amoxicillin; ATM = aztreonam; CAZ = ceftazidime; CFX = cefalexin; CIP = ciprofloxacin; CPO = cefpodoxime; CTX = cefotaxime; CXM = cefuroxime; ERT = ertapenem; GEN = gentamicin; MEC = mecillinam; 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.

P679 Anti-pseudomonal activity of piperacillin/tazobactam: more than a decade of experience from the SENTRY Antimicrobial Surveillance Program (1997–2007)

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Objectives: To summarise the susceptibility rate (% susceptible [S]) experience for piperacillin/tazobactam (P/T) tested against *Pseudomonas aeruginosa* isolates from the Asia-Pacific (APAC), Europe (EU), Latin America (LA) and North America (NA) for 1997–2007. All testing was by reference CLSI (2006) methods and interpreted by contemporary CLSI and USA-FDA breakpoint criteria (2008).

Methods: A total of 25,460 *P. aeruginosa* were tested originating from APAC (4,441), EU (7,695), LA (4,277) and NA (9,047); >110 medical centres/year and samples averaging >30 nations/year. CLSI M7-A7 (2006) and M100-S18 (2008) methods and categorical criteria were applied and all quality control results were within published limits. For this

analysis results from 1997–2007, 1997–1999, 2005–2007, APAC, EU, LA and NA were assessed against several broad-spectrum beta-lactams (cefepime [CPM]), ceftazidime [CAZ], imipenem [IMP], meropenem [MER], and piperacillin alone [PIP]; total of 12 agents overall.
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%) 7> 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% S), 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 LA isolates. In contrast, ticarcillin/clavulanate S rates were lower than ticarcillin tested alone in NA (−1.5%; antagonism) and this agent only inhibited 70.3% of isolates worldwide.

Table

Beta-lactam	% susceptible by region (no. tested)				
	APAC (4,441)	EU (7,695)	LA (4,277)	NA (9,047)	All (25,460)
P/T	82.9	83.0	74.8	88.7	83.6
MER	83.5	81.5	71.6	89.4	83.0
IMP	80.4	78.0	68.7	85.9	79.7
PIP	79.3	78.4	67.7	86.1	79.5
CPM	77.0	77.1	64.6	84.0	77.5
CAZ	74.7	75.9	62.8	82.4	75.8

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 at >90%. R surveillance programs should be sustained to document emerging patterns of old and newer agents for difficult to treat pathogens such as *P. aeruginosa*.

P680 ECO-SENS II: antimicrobial susceptibility in *Escherichia coli* from community acquired urinary tract infections in Austria, Greece, Portugal, Sweden and United Kingdom

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Objectives: The original ECO-SENS international survey of prevalence of pathogens and antimicrobial resistance in uncomplicated urinary tract infections in women was performed in 1999 to 2000 (Journal of Antimicrobial Chemotherapy 46 S1:15–22 (2000) and 51:69–76 (2003)). To compare resistance development over time, the ECO-SENS II, performed in 2007–2008, determined the antimicrobial susceptibility from similarly acquired *E. coli* in 5 of the countries.
Method: Five European countries (see title) where chosen to represent different areas of Europe. The aim was to collect 200 *E. coli* from each country. Susceptibility tests were performed in accordance with SRGA recommendations (www.srga.org) on 14 antimicrobials.

Table 1. Resistance among *E. coli* from urinary tract infections in five European countries in 2008 compared to resistant rate in the first ECO·SENS-study in 2000

Country	Rate for ECO·SENS II (for ECO·SENS I), %													
	AMP	AMC	MEC	TRI	SUL	TSU	NIT	NA	CIP	GEN	FOS	CDR	CTX	CAZ
Austria	30.0	10.0	0	16.4	20.9	14.5	0.9	9.1	4.5	1.8	0.9	4.5	2.7	2.7
	(17.5)	(2.4)	(1.6)	(9.5)	(25.4)	(9.5)	(0.8)	(2.4)	(0)	(0.8)	(0)	(0.8)	(NT)	(NT)
Greece	25.8	4.2	1.4	18.8	23.0	17.8	0	13.1	5.6	0.9	2.8	1.4	1.4	0.5
	(22.0)	(0.8)	(0.8)	(13.6)	(19.7)	(11.4)	(3.0)	(6.8)	(1.5)	(0.8)	(1.5)	(3.0)	(NT)	(NT)
Portugal	35.0	4.9	0	17.6	31.4	16.7	0	13.7	6.9	2.9	0.9	0	0	0
	(45.3)	(9.3)	(2.3)	(26.7)	(44.2)	(26.7)	(5.8)	(11.6)	(5.8)	(3.5)	(0)	(2.3)	(NT)	(NT)
Sweden	21.0	2.4	0.5	16.1	22.4	16.1	0	6.3	2.4	1.5	1.0	1.5	1.5	1.0
	(15.5)	(5.7)	(1.6)	(8.8)	(16.6)	(8.3)	(0)	(2.6)	(0)	(0)	(0.5)	(5.2)	(NT)	(NT)
UK	31.9	2.0	1.0	15.2	26.5	14.7	0	7.4	1.0	0.5	0.5	1.5	0.5	0.5
	(37.2)	(2.8)	(1.7)	(13.3)	(37.7)	(12.2)	(0)	(2.2)	(0.6)	(0)	(0)	(1.7)	(NT)	(NT)
Total (n = 834)	27.8	4.1	0.7	14.6	20.9	16.1	0.1	9.6	3.7	2.6	1.3	1.7	1.2	0.8

Results: To date 834 *E. coli* have been studied (at least 150 per country). The results are summarised in Table 1. Among those we found six *E. coli* to be ESBL producers, as compared to none in the first ECO-SENS survey. In 37 of 60 instances, resistance rates were higher in 2008 than in 2000. In five countries ciprofloxacin resistance and in 4 trimethoprim resistance had increased. Resistance to pivmecillinam, nitrofurantoin and fosfomycin was low in all countries.
Conclusion: Antimicrobial resistance in community acquired *E. coli* continues to increase. This was especially true for fluoroquinolone and trimethoprim resistance. However, resistance to pivmecillinam, nitrofurantoin and fosfomycin was low in all countries and had with few exceptions not increased between 2000 and 2008.
Both ECO-SENS surveys were sponsored by LEO Pharma, Ballerup, Denmark.

P681 Catheter-associated urinary tract infections in a geriatric ward

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Objectives: Catheter-associated urinary tract infections (CA-UTIs) in the elderly represent the most common nosocomial infections; about 15 to 30% of hospitalised elderly patients with acute conditions usually undergo urinary catheterisation. This study was done to describe the incidence of catheter-related infections, the risk factors associated with the duration of catheterism and the microbiological pathogens involved.
Methods: An active surveillance system of nosocomial infections associated with CA-UTIs in patients hospitalised in the geriatric ward of a country hospital (720 beds) was carried out from January 2006 to December 2007, and a study of prevalence was made in July 2008. The surveillance was based on CDC's Guideline for prevention of Catheter-associated Urinary Tract Infections. Standard clinical and microbiological criteria were used to define colonisation and infection.
Results: A total of 122 patients, negative for bacteriuria, underwent urinary catheterism during the observation phase. The incidence rate/100 catheter days was 7.09% for 57 cases of infections. The average days of catheterisation was 6.6 days. We calculated the average catheter-days of the infections for each year of the study; the results were 10.28 and 17.42, with a significant correlation between the infection and the catheter days. The percentage of catheterisation was 12.88%. The study of prevalence made in July 2008 confirmed the previous data: the prevalence rate of infections/catheter-days was 8.3%, the percentage of patients undergoing urinary catheter was 25%. The bacterial aetiology of CA-UTIs generally involved a single uropathogen, mainly E. Coli, but several other bacterial pathogens and yeasts were detected: *P. mirabilis*, *K. pneumoniae*, *E. faecalis*, *Citrobacter* spp., *P. stuartii*, *M. morganii*, and *Candida* spp.
Conclusions: Urinary catheters are used frequently in elderly populations. The infection rate is about 7–8% per day. *Escherichia coli* remains the most common infecting organism, but a wide variety of other organisms may be isolated, including yeast species. The duration of catheterism is a confirmed risk factor for developing CA-UTIs.

P682 Detection and management of catheter-associated urinary tract infections: an audit of clinical practice in a district general hospital

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Objectives: Urinary catheters introduce a portal for invasion by infectious organisms, resulting in catheter associated urinary tract infections (CAUTI), the source of 8% of hospital acquired bacteraemia. North Middlesex University Hospital, London, UK guidelines on managing CAUTI indicated the need for catheter change and sensitivity-guided antibiotic usage, but did not focus on distinguishing between asymptomatic bacteriuria and septic patients. Thus, a clinical audit was carried out to identify clinical parameters involved in the detection and management of microbiologically confirmed catheter associated bacteriuria.
Methods: Patients were identified from catheter specimen urine (CSU) that had grown a single organism $>10^5$ cfu/ml. Their medical records

were then analysed to assess the parameters being audited: the indication for CSU collection (clinical suspicion, costovertebral 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, neutropaenia). 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.

P683 Changing trends in community urinary tract infections in South Manchester

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Objectives: Urinary Tract Infections (UTIs) are the second most common clinical indication for empirical antibiotic treatment in primary and secondary care. Many different antibiotic resistance mechanisms are present, but the mechanism of extended-spectrum beta-lactamase (ESBL) production is of particular interest. Periodic assessment of the causative organisms for UTIs allows clinicians to keep a track of species and any changes in the trends of bacterial susceptibility. We aimed to look at the patterns of organisms and antibiotic sensitivities for all urinary isolates from patients in the South Manchester community, over a four year period (2004–2007).
Methods: Data was collected retrospectively from the microbiology laboratory database (Telepath) at our hospital. Analysis of the positive isolates received over the four year period was performed using Microsoft Excel.
Results: The majority (>80%) of bacterial species responsible for UTIs were Gram-negative. The approximate ratio of 5.4:1 between Gram-negative and Gram-positive species was maintained for the entire period. For each year the main Gram-negative species were enterobacteriaceae, while enterococci were the most common Gram-positive species. Overall antibiotic sensitivity has declined. Trimethoprim (6% decrease) and ciprofloxacin (5% decrease) have shown the largest reduction followed by amoxicillin, cephalexin and piv-mecillinam with decreases of 2–3% each. Sensitivity to nitrofurantoin has remained largely unchanged. The proportion of UTIs due to ESBL producers has increased annually, showing a more than threefold increase from 2004–07. The majority of ESBL producers are *Escherichia coli*. Sensitivity to nitrofurantoin and piv-mecillinam has remained high (around 90%) with no notable change for carbapenems.
Conclusions: During the period 2004–07, there has been an increase in antibiotic resistance to the first line antibiotics used by general practitioners to treat urinary tract infections in South Manchester. There has been an increase in ESBL incidence which may be direct result of antibiotic use or a spillover of nosocomial organisms into the community. Prudent antibiotic prescribing is generally recommended to combat increasing antibiotic resistance. The increased use of nitrofurantoin and piv-mecillinam as empiric agents for community UTI may be considered. It is worth expanding the scope of the study to include data from 2008. We hope to include these data in any future presentations.

P684 Persistence of *Escherichia coli* clones in recurrent urinary tract infections

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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 Køge, Denmark. Of these, 41 isolates were 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 (kpsM 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 patients 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.

P685 Antibiotics versus placebo in the treatment of women with uncomplicated cystitis: a meta-analysis of randomised controlled trials

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Objective: Acute uncomplicated cystitis is one of most common bacterial infections in women and is conventionally treated with antibiotics. However, emergence of resistant uropathogens forces physicians to reconsider the prescription of antibiotics for acute uncomplicated cystitis in non-pregnant young women. We aimed to evaluate the effectiveness of antibiotics in the treatment of acute uncomplicated cystitis.
Methods: We searched PubMed, the Cochrane Central Register of Controlled Trials and Scopus database.
Results: Five randomised controlled trials (RCTs) involving non-pregnant, non-immunocompromised adult women with clinically and microbiologically documented acute uncomplicated cystitis were included. Clinical success was significantly more likely in women treated with antibiotics versus those treated with placebo [4 RCTs, 1062 patients, random effects model (REM), odds ratio (OR) = 4.81, 95% confidence intervals (CI) = 2.51–9.21]. Antibiotics were also superior to placebo, regarding cure (4 RCTs, 1062 patients, REM, OR = 4.67, 95%CI = 2.34–9.35); microbiological eradication at the end of treatment (3 RCTs, 967 patients, REM, OR = 10.67, 95%CI = 2.96–38.43); after the end of treatment (3 RCTs, 738 patients, REM, OR = 5.38, 95%CI = 1.63–17.77), and microbiological reinfection or relapse (5 RCTs, 843 patients, REM, OR = 0.27, 95%CI = 0.13–0.55).

However, adverse events were more likely to occur in antibiotic-treated patients versus placebo-treated women (4 RCTs, 1068 patients, REM, OR = 1.64, 95%CI = 1.10–2.44). No difference was found between the compared treatment arms regarding study withdrawals from adverse events, the development of pyelonephritis and emergence of resistance.
Conclusion: Antibiotics are superior to placebo regarding both clinical and microbiological success in adult non-pregnant women with microbiologically confirmed acute uncomplicated cystitis. However, they are associated with more adverse events.

P686 An analysis of isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from genital tract specimens of women receiving care at a general hospital serving rural population in Greece

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Objective: *Mycoplasma hominis* and *Ureaplasma urealyticum* are among the most common microorganisms isolated from the genital tract of sexually active women. Furthermore, some experts perform screening for *M. hominis* and *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 *M. hominis* and *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 *M. hominis* and/or *U. urealyticum*.
Results: The overall frequency of isolation of *M. hominis* and *U. urealyticum* in pregnant women (n = 528) was 2% and 14%, respectively. The overall frequency of isolation of *M. hominis* and *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 *M. hominis* and *U. urealyticum* isolation are within the range of the figures reported in studies performed in various parts of the world.

P687 Symptomatic women with non-gonococcal, non-chlamydial cervicitis show a high incidence of *Mycoplasma genitalium* in Greece

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Objectives: The purpose of the present prospective study was to assess the incidence of *Mycoplasma genitalium* (*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 nongonococcal, nonchlamydial 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 37ºC for 24 hours, whereas anaerobic cultures were carried out on Wilkins-Chalgren agar at 37ºC for 48 hours. The isolated strains were identified using the automated system VITEK 2 (BioMerieux, France). For the identification of *Ureaplasma urealyticum* and *Mycoplasma hominis* we used *Mycoplasma* IST 2 (BioMerieux, France). Samples were tested for *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: *M. genitalium* was detected in 27 (18%) of the 150 women tested. Data regarding women tested positive for *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 *M. genitalium* isolated from their cervices (p = 0.021). Pruritus, in contrast to other symptoms, was more frequently associated with *M. genitalium* cervicitis (p = 0.05) while dyspareunia was significantly increased in the control group (p = 0.05) where other pathogens except *M. genitalium* where isolated. In 9 patients *M. genitalium* was the only pathogen isolated while in 12 cases was isolated together with *Ureaplasma urealyticum*. Finally, 6 patients presented with infection by all 3 mycoplasmas tested.
Conclusions: Symptomatic women harbour *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.

P688 Anorectal *Chlamydia trachomatis* infections in Swiss HIV-infected homosexual men

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Objectives: Since 2003, anorectal lymphogranuloma venereum (LGV) and non-LGV *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).

Table 1. Characteristics of 147 male homosexual participants in the Swiss HIV Cohort Study

	No. (%) *C. trachomatis*-positive patients (n = 16)	No. (%) *C. trachomatis*-negative patients (n = 131)	p-value[1]
Age (median years, IQR)	40.5 (34-47)	43 (38-45)	0.27
CD4 cell count (cell/mm³)*			0.57[2]
<200	1 (6)	3 (2)	
200-499	7 (44)	70 (55)	
>499	8 (50)	54 (43)	
HIV-1 RNA < 40 copies/ml*	11 (69)	74 (59)	0.44
Drug use[3]	5 (31)	37 (28)	0.8
Alcohol misuse[4]	5 (31)	51 (39)	0.6
Hepatitis C antibodies*			0.36
Positive	2 (13)	10 (8)	
Negative	14 (93)	117 (94)	
Hepatitis B core antibodies*			0.96
Positive	7 (47)	59 (46)	
Negative	8 (53)	69 (54)	
Concurrent proctitis	3 (19)	23 (19)	1
Anal *N. gonorrhoeae* PCR positive	0	1	
Concurrent urogenital infection	1 (6)	12 (9)	1
Previous chlamydial infection	4 (25)	31 (24)	1
Previous gonorrhoeal infection	5 (31)	58 (44)	0.42
Previous syphilis infection[5]	9 (56)	55 (42)	0.28
Previous urethritis of unknown origin	0	1	
No. sexual partners within last 2 years			**0.001**
≤ 20	8 (50)	96 (85)	
> 20	8 (50)	17 (15)	
HIV partner(s) within last 2 years			0.29
Yes	14 (88)	86 (66)	
No	2 (12)	39 (30)	
Condom use with occasional partners[6]			0.7
Never/rarely/sometimes	7 (47)	36 (41)	
Always	8 (53)	51 (59)	
Insertive anal*	11 (69)	79 (62)	0.51
Insertive oral*	13 (81)	84 (64)	0.26
Insertive vaginal*	1 (6)	4 (3)	0.44
Receptive oral*	14 (88)	102 (78)	0.52
Fist*	1 (6)	15 (11)	1
Anal toys*	7 (44)	33 (25)	0.12
Rimming*	9 (56)	71 (54)	0.88
Had been paid for sex*	1 (6)	14 (11)	1

[1]Fisher's exact *p*
*Data missing for few cases
[2]< vs. >= 500 cell/mm³
[3]Defined as injection or non-injection drug use within the last 6 months
[4]Defined as a daily consumption above 30g of alcohol
[5]Defined as a positive Treponema Pallidum Haemagglutination Assay
[6]n=102, within the past 6 months

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 viraemia >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.

P689 Characteristics of patients infected with *Mycoplasma genitalium* in an STI outpatient clinic, Rotterdam, the Netherlands

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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 *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%) no history of other STIs. Twenty-nine (69%) reported no symptoms and 10 (24%) one or more symptom, 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.

P690 Micro-organisms isolated from male urethral exudates during an 8-year period in a Spanish teaching hospital

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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 37ºC in O2, 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 antimicrobian agents studied, increased considerably during the period.
Conclusions: According to positive cultures, the most prevalent microorganism isolated from urethral exudates was Neisseriae 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.

P691 Seroepidemiological survey of viral hepatitis, HIV, syphilis and herpes simplex virus in a Portuguese correctional facility

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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 ("chemiluminescent microparticle immunoassay") 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.

P692 Prevalence of syphilis in volunteer blood donors in a defined area of northern Greece: a retrospective study (1998–2008)

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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 comercial 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 (THPA) and the fluorescent treponemal antibody absorbtion test (FTA-ABS).
Results: During the study only one serum sample was found RPR-positive. The diagnosis of syphilis was confirmed by both THPA 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

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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

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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 persons 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 ($\leqslant$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

interval, 2.66–12.3), while HIV infection was of borderline statistical significance in association with JH reaction (OR 2.30, 95% CI 0.87–6.06, and p = 0.09).
Conclusion: Non-latent syphilis was associated with higher risk for JH reactions in persons who received penicillin therapy according to treatment guidelines. A larger sample size of subjects is needed to confirm if HIV infection is an independent factor for development of JH reaction.

P695 Evaluation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in patients visiting gynaecology department of hospitals in Delhi using an in-house PCR assay and ELISA-based method of detection

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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%), with high prevalence in females with history of ectopic pregnancy and primary infertility. Using Roche Amplicor Micro well plate test as gold standard, the sensitivity and specificity of the in-house PCR assay was found to be 93% and 97.45%, for CT, while it was 88% and 82% respectively for NG. Use of molecular probes further increased the sensitivity of the assay and reduced the time for analysis making it a rapid point of care test. An ELISA based detection assay was also developed using purified proteins of NG and CT cloned in bacterial expression vector. Serum samples of the patients and healthy volunteers were checked for determining the specificity and sensitivity and validated by PCR assay of endocervical swabs.
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.

P696 Azithromycin-resistant *Neisseria gonorrhoeae* strains recently isolated in Italy

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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 $\geqslant$ 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. 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.

P697 Multiple mutations and change in conformation of Mtr efflux pump leading to penicillin resistance in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae, (NG), facultative intracellular pathogen causes, gonorrhoea, a sexually transmitted disease. Strains resistant to penicillin, a common drug used in developing countries for its treatment, have become prevalent world wide. This is attributed to presence of beta-lactamase encoding plasmids (PPNG strains) or active removal of the drug due to increased expression of the MtrCDE efflux pump (chromosomal mediated resistance). The expression of efflux pump is negatively controlled by MtrR protein. Mutations in MtrR or in the promoter region of Mtr operon lead to increased expression of the efflux pump (MtrCDE). This leads to increased drug resistance among NG strains. Mutations in MtrE have also been shown to be associated with increased resistance towards hydrophobic drugs.
Objectives:
a. Analysis of mutations in MtrR and MtrE genes of penicillin resistant clinical isolates.
b. Structure analysis of MtrE and MtrR in context of mutations.

Methodology:
a. PCR amplification and sequencing of the amplicons of MtrR and MtrE genes from penicillin resistant clinical isolates.
b. Mutation analysis of amplicons using BLAST and ClustalW.
c. Structure analysis of MtrE and MtrR using Insight II program.

Results: Mutations were found in helix turn helix motif of MtrR (L33V, G45D) and in its C′ terminal (Y105H) region. Mutant MtrR protein shows reduced binding affinity to its promoter leading to increased expression of MtrCDE efflux pump thereby causing resistance to penicillin and other hydrophobic drugs. Analysis of nucleotide sequence of MtrE also showed distinct mutations (I429S, K165E, K191R, and R285G) in different clinical isolates with K191R as the most frequent mutation observed. Strains with mutations in both MtrR and in MtrE showed higher MIC values (16 to 22 microgram/ml). PPNG strains with mutant MtrR or MtrE had even higher MIC for penicillin (32 microgram/ml). In-silico modeling of MtrE protein indicates that mutations (K191R and R285G) in MtrE distorts hydrogen bonding and thus results in structural changes which may be responsible for altering efflux activity.

Conclusion: Mutations at multiple loci act in synergism with each other to confer high penicillin resistance to NG strains.

P698 Screening of macrolide resistance locus in *Treponema pallidum* subsp. *pallidum* in the Czech Republic

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Objectives: Due to alergies to penicillin, macrolide regimens are frequently applied in the treatment of syphilis. Unlike penicillin, macrolide treatment represents a risk of treatment failure due to chromosomally encoded resistance in *Treponema pallidum* subspecies *pallidum*. A2058G mutation in the 23S rDNA locus was described to cause erythromycin resistance in syphilis. Molecular detection of syphilis represents the only available test to reveal macrolide resistance in clinical isolates, because *T. pallidum* can not be cultured in vitro. Our aim was to assess the incidence of this mutation among clinical isolates in the Czech Republic.
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 rRNA gene was developed and direct Sanger sequencing of PCR products was applied to detect mutations in corresponding part of the 23S rRNA 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 rRNA 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 alergic patients.
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P699 Prevalence and antimicrobial susceptibilities of genital mycoplasmas in outpatients with clinical urogenital infections in Korea

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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).
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 *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

P700 Evaluation of EDTA and dipicolinic acid, with and without the addition of zinc, in the detection of metallo-beta-lactamases

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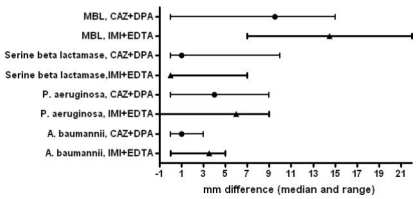
Objectives: Carbapenem-resistant Gram-negative bacteria carrying transferable resistance genes are spreading worldwide, and to stop local epidemics we must be able to detect them. Phenotypic detection of carbapenemases belonging to the metallo-beta-lactamases (MBL) is problematic. We compared the performance 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 mg/l of ZnSO4x7H2O. 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-betalactamases: zone diameter differences between imipenem vs. 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+zinc 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.

Conclusion: Zone mm difference comparison of IMI and IMI+EDTA was found to be the most sensitive method. Sensitivity of DPA was low due to the small CAZ zones of MBL strains. Zinc improved only the *P. aeruginosa* results.

P701 Evaluation of drug-drug interaction study of zabofloxacin in vivo

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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-inflammtory 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 wafarin, the PT was determined at 24 hrs after FQ dosing.
Results: Gati, levo 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.

P702 In vitro antibacterial and anti-pathogenic activity of colistin, azithromycin and their combinations against colistin-susceptible and -resistant *Pseudomonas aeruginosa*

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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 PAO1 and 3 clinical isolates (COL^S, MIC $\leqslant$ 2 mg/L) and 4 COL resistant isolates (COL^R, 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^S. For COL^R, 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 24 h or 48 h depending on the isolate. Antibacterial effect was measured by viable counting. Synergy was regarded as fractional inhibitory concentrations $\leqslant$ 0.5. Analytical methods based on LCMS and HPLC were developed to assay the quorum sensing molecule N-3-oxododecanoyl-homoserine lactone (C12-HSL) and pyocyanin, respectively (limits of quantification 0.5 mg/L and 0.2 mg/L, respectively). Biofilm was assayed using crystal violet.
Results: Synergy was bidirectional. A marked synergy was observed against COL^R, 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^R. 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^S; AZM effect was minimal. In general, COL^R 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.

P703 Interactions between linezolid and a carbapenem on methicillin-resistant *Staphylococcus aureus*

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is both a therapeutic and epidemiologic 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 meropemem and ertapenem 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 microlitres 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 carbapemens 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 metabolisms, 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.

P704 In vitro synergistic activity of ceftriaxone, rifampicin and doxycycline against *Brucella melitensis* isolates by E-Test

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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 (67%) 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 doxycyclin as a therapeutic alternative for neurobrucellosis.

P705 In vitro activity of polymyxin B and rifampicin in combination against pandrug-resistant *Acinetobacter* spp.

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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 10^5 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 amplication 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 PDR 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 gentamycin against *Enterococcus faecalis*: interaction studies using a calorimetry assay

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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 5×10^5 cfu/ml of the test strain. Heat generation of bacterial culture without antibiotic was used as control. Ampoules were air-tightly sealed and heat production of the growing cultures at 37ºC 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 ug/ml for VAN, 1 ug/ml for DAP and 8 ug/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 subinhibtory 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.

DAP (×MIC)	GEN (×MIC)	Peak heat flow	VAN (×MIC)	GEN (×MIC)	Peak heat flow
0	0	100%	0	0	100%
0.5	0	59%	0.5	0	88%
0.5	0.125	54%	0.5	0.5	66%
0.5	0.062	58%	0.5	0.25	84%
0.25	0	75%	0.25	0	98%
0.25	0.125	72%	0.25	0.5	89%
0.25	0.062	74%	0.25	0.25	99%

P707 Evaluation of the in vitro activity of tigecycline alone and in combination with rifampicin against multidrug-resistant Gram-negative bacilli

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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 (ESBls, 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 $\leqslant 0.5$), addition ($0.5 < \text{FIC} \leqslant 1$), indifference ($1 \leqslant \text{FIC} < 2$) and antagonism (FIC $\geqslant 2$). 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*

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Objectives: *Clostridium difficile* is an important cause of hospital acquired diarrhoea (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 10^5 cfu/ml on Brucella agar containing 5% sheep blood. Following incubation (anaerobic), the lowest drug concentration preventing growth was the MIC. For mutant prevention concentration (MPC) testing, $\geqslant 10^9$ CFUs were applied to Tryptic soy agar plates (TSA) containing 5% sheep red blood cells containing doubling drug dilutions and following incubation (anaerobic), the lowest concentration preventing growth was the MPC.
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 9689 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 9689 were 0.125 and 2 mg/L respectively. MIC and MPC values for Tig against ciprofloxacin resistant strain (MIC $\geqslant$ 32 mg/L) were 0.047–0.094 and 0.063 mg/L respectively; 0.38–1.5 and 2-mg/L respective for vancomycin.
Conclusions: TIG was highly active in vitro against contemporary CD isolates with MIC and MPC values $\leqslant$ 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*

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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 $\geqslant 10^9$ 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 10^5 cfu/ml tested against doubling drug dilutions in Todd-Hewitt broth with incubation at 35–37 degrees Celsius in 5% CO2 for 18–24 hours. For MPC testing, $\geqslant 10^9$ 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 $\geqslant$ 128 mg/L had a CET MPC of $\geqslant$ 2 mg/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 Comparison of modified microbroth dilution to agar dilution for determining the mutant prevention concentration of gatifloxacin and moxifloxacin against *Streptococcus pneumoniae* ATCC 49619

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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, $\geqslant 10^9$ CFUs of SP ATCC 49619 were inoculated to drug containing agar plates and incubated under ideal conditions (5% CO2 at 35–37 Degree Celsius) for 24–48 hours. For the modified microbroth dilution, 10^1-10^7 cfu/ml were added to microtiter wells containing media and doubling drug dilutions. Following incubation under ideal conditions, the lowest drug concentration preventing growth was recorded as the MPC.
Results: MPC values for Gfx and Mfx by agar dilution was 0.5 mg/L. For Gfx, MIC (MPC) values for the 10^1-10^4 cfu/ml inocula was 0.125 mg/L, 0.25 mg/L for the 10^5-10^6 cfu/ml inocula and 0.5 mg/L for the

10^7 cfu/ml inocula. For Mfx, MIC (MPC) values were 0.063–0.125 mg/L for 10^1-10^4 cfu/ml, 0.125 mg/L for 10^5 cfu/ml, 0.25 mg/L for 10^6 cfu/ml and 0.5 mg/L for 10^7 cfu/ml. These values were reproducible when the assays were repeated severe times.
Conclusion: Using a modified microbroth dilution method gave MPC results that were consistent with the established agar dilution method for testing SP ATCC 49619 against Gfx and Mfx. This observation may have important implications for the advancement of MPC susceptibility testing and may facilitate the introduction of this testing to a larger number of clinical laboratories.

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

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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 (bioMerieux, Marcy l'Etoile, France). Finally, IgG Avidity was performed by Vidas Toxo IgG Avidity (bioMerieux, 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 1. Enzygnost system for IgA anti-Toxoplasma and Enzywell Toxoplasma IgA reactivities in relation to the four groups of subjects tested for the comparative study

Study group[a]	No. of samples	Number of reactive samples when tested by Enzygnost and Enzywell, respectively (%)				% Concordance
		+ and +	− and −	+ and −	− and +	
Pregnant women	57	41 (71.3)	8 (14.0)	7 (12.3)	1 (1.8)	86.0
Infants	32	0 (0)	32 (100)	0 (0)	0 (0)	100
HIV positive	26	3 (11.5)	22 (84.6)	0 (0)	1 (3.8)	96.2
Healthy blood donors	57	0 (0)	54 (94.7)	0 (0)	3 (5.3)	94.7
Total	172	44 (25.6)	116 (67.4)	7 (4.1)	5 (2.3)	93.0

[a] Immunological situation of the patients:
- All the pregnant women 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

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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 DALYs with a range of 818–6713 DALYs. In the previous calculations using the incidence of the Dutch TIP study of 1987 this was 620 (range 220–1900) DALYs.
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

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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

tissue tropism. Bbss causes most of the Lyme arthritis cases, while Bg is associated with neuroborreliosis and Ba with chronic skin disorders. Decorin binding proteins of these three genospecies differ in their amino acid sequence, but little attention has been paid to the potential difference in their biological activity.
Methods: We have constructed three recombinant *Borrelia* strains through cloning of dbpAB operons from Bbss, Bg and Ba to the non-infective Bbss B313 strain, which lacks several major surface proteins including DbpA and B. dbpAB operon was PCR amplified from genomic DNA of Bbss N40, Bg SBK40 and Ba A91 strains, cloned to pBSV2 shuttle vector, and electroporated into B313. The binding of biotinylated decorin to the recombinant *Borrelia* strains was studied by Western blot and dot blot assays. In cell adhesion studies, the binding of fluorescent recombinant *Borrelia* strains to fibroblasts was studied using confocal microscopy. Also, we have expressed individual recombinant DbpA and B of the three genospecies in *E. coli*.
Results: The strains expressing Dbps from Bbss and Bg bound to decorin and decorin expressing fibroblasts, while the strain with Dbps from Ba showed only little adherence to decorin and fibroblasts. Analysis of the binding properties of the individual Dbps expressed in *E. coli* is underway.
Conclusions: DbpA and/or DbpB from Bbss and Bg mediate adherence of the recombinant bacteria to purified decorin and to cells expressing decorin. In contrast, the Dbps from Ba do not have this activity.

P714 Patients after proven neuroborreliosis – how severe is the persisting neuropsychological damage?

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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 Scripps 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 questionnaire 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.

P715 Molecular analysis of *Borrelia spirochetes* detected in *Ixodes granulatus* ticks removed from rodents in Taiwan

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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 specimen 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 amplicon 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.

P716 Performance of four commercial immunoblots for serological confirmation of Lyme borreliosis in Rhone-Alpes (France)

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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), Ecoblot® (Virotech, Meridian), EU Lyme WB® (MarDx, Trinity Biotech) and Recomblot® (Mikrogen, Diasorin). 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®, Ecoblot®, 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®, Ecoblot®, 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). The diagnosis sensitivity evaluated for Europe Line®, Ecoblot®, EU Lyme WB® and Recomblot® were 59.5%, 64.3%, 54.8%, 66.7% for IgG respectively and 59.%, 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.

Discrepancies in finding obtained on identical sera indicate the need for standardisation of the serological methods for confirmation LB infection. A good confirmatory test requires a high specificity evaluated among the population followed.

Immunology, host defences and immunotherapy

P717 The polycyclic aromatic hydrocarbons modify response of lung epithelial cells to *Aspergillus fumigatus* spores, in vitro

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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% fenantrene 30% acenaphtene 9% fluorene and acenaphtene, fluoranthene 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.

P718 Echinaceae purpureae herba inhibits adhesion and invasion of *Streptococcus pyogenes* adhering to and invading human mucosal epithelial cells

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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 (Echinaceae purpureae 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).
Conclusions: The Echinaceae purpureae 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.

P719 Potentially probiotic bacteria induce inflammasome activation and cytokine production in human monocyte-derived macrophages

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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-1b production, and whether suppressor of cytokine signaling (SOCS3)-mediated negative feed-back 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-1b, 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 feed back 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-1b 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.

P720 Toll-like receptor ligand induced synergistic interferon gene expression in human monocyte-derived dendritic cells

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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

factor and interleukin 4. DCs were stimulated with TLR3 ligand polyI:C, TLR4 ligand LPS and TLR7/8 ligand R848 alone or in a combination of two. RNA was isolated for semi quantitative RT-PCR analysis. DNA binding assay was used to study TLR ligand induced binding of transcription factors to the IFN-lambda 1 promoter IFN stimulated response element (ISRE).
Results: Stimulation of DCs with polyI:C or LPS alone induced mRNA expression of IFN-β, IFN-λ1 and IFN-λ 2/3, whereas R848 alone was ineffective. However, simultaneous stimulation with R848 together with polyI:C led to a synergistic IFN-α, -β, -λ 1 and -λ 2/3 mRNA expression already in 3 h and the synergistic effect was sustained in the later time points. Interestingly, R848 combined with LPS induced synergistic IFN mRNA expression only at the later time points, when the IFN expression induced with LPS alone had declined. To clarify the mechanisms behind the synergistic effect, DNA binding assay was used to study LPS or/and R848 induced binding of IFN regulatory factor (IRF) and signal transducer and activator of transcription (STAT) transcription factors to the IFN-λ 1 promoter ISRE site. LPS and/or R848 stimulation induced the binding of IRF1, IRF7 and IRF8 to the ISRE site while IRF4 and IRF5 were bound constitutively. Only LPS was able to induce binding of STAT1 and STAT2 to the ISRE site.
Conclusion: This data suggest that TLR3 or TLR4 ligand combined with TLR7/8 ligand induces synergistic and sustained IFN expression in human monocyte derived DCs. However, there might be differences in the pathways driving the synergistic IFN response, as its kinetics and magnitude varied between the two ligand combinations. Understanding the molecular mechanisms behind the synergistic IFN response is needed when designing better vaccine adjuvants or DC-based therapies.

P721 Detection of HIV-specific cytotoxic T-lymphocytes using viral delivery system

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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-γusing 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 suported in part by AIDS Trust Fund, Hong Kong.

P722 Neuropeptide S receptor 1 variation is associated with *Chlamydia pneumoniae* seropositivity in Finnish military conscripts

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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, GPRA) 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 $\geqslant$ 128 and/or IgA titre $\geqslant$40) both at entry (n = 144, 16%) and at end (n = 91/790, 12%) of the service. In a haplotype specific logistic regression analysis, H2 was found to be a risk haplotype, OR = 1.46 and p = 0.010 at arrival, and OR = 1.45 and p = 0.037 at leave, whereas H1 was found to be a protective haplotype, OR = 0.74 and p = 0.036 at arrival, and OR = 0.61 and p = 0.005 at leave. The analyses were adjusted for asthma, duration of the service, and intake group. No association was seen between NPSR1 haplotypes and asthma, or the presence of *C. pneumoniae* IgM and/or IgG or IgA seroconversions during the service.
Methods: Our study population consisted of 226 asthmatic and 659 non-asthmatic men from two intake groups who were at military service in the Kainuu Brigade between July 2004 and January 2006. Serum samples were obtained at the beginning and at the end of the service, and during infection episodes. Serum *C. pneumoniae* IgG, IgA, and IgM antibodies were measured by a microimmunofluoressence method. Seven tagging single nucleotide polymorphisms in the conserved haplotype block of NPSR1 were genotyped on a MALDI-TOF platform. Statistical analysis was performed with PLINK software version 1.01.
Conclusion: Variation in NPSR1 gene may affect the susceptibility to *C. pneumoniae* infection. Association was observed between the haplotypes and high IgG and/or IgA antibody levels both at entry and at leave of the service, but not for markers of acute infection. This may imply that NPSR1 plays a role especially in persistent *C. pneumoniae* infection. No association was seen between NPSR1 variation and asthma, possibly since there are no severe asthma cases in our study population. Further studies are needed to address the question if *C. pneumoniae* infection modulates the effect of NPSR1 variation on asthma susceptibility.

P723 IgG-antibody response against *Clostridium difficile* antigens, PCR-ribotype and relapse of *C. difficile*-infection

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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 027 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

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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 H/L, X/Y and P/Q) 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. SPSS 15.0 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 $\geqslant 30.0$). 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 combined overweight and obese group (BMI $\geqslant$ 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 $\geqslant$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 IFNGR1 promoter is correlated to the susceptibility to chronic HBV infection in Chinese population

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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 signaling transduction pathway is directly controlled by the IFNGR1 expression level. In our study, single nucleotide polymorphisms (SNPs) in the IFNGR1 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 IFNGR1 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 IFNGR1 promoter with the –56C is significantly higher than that with –56T.
Conclusion: –56C/T SNP in IFNGR1 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

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Objectives: Aim of present study was comparison of safety and immunoactivity 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 substrain), Onko BCG 50 (Biomed Lublin, Poland) preparation (Brazylian Moreau substrain).
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
Multiplation 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 1.8×10^6 CFU/spleen in mice vaccinated with Tice BCG and 1.1×10^6 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

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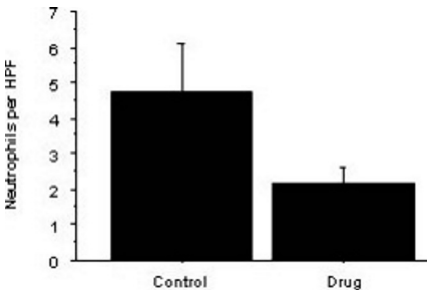
Objectives: Quinolone antibiotics concentrate in inflammatory cells and may be immunomodulatory. We hypothesised that oral moxifloxacin

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 (ng/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.



P728 **Adenosine A2A receptor agonist (2-chloroadenosine) treatment modulates pro-inflammatory immune response during the course of *Klebsiella pneumoniae* B5055-induced acute lung infection in BALB/c mice**

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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-1a and IL-10 levels 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-1a, 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-1a, 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.

P729 **Interaction with complement factor H and C4b-binding protein contributes to the serum resistance of an anaerobic pathogen, *Fusobacterium necrophorum***

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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 C4b 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.

P730 **Action of bacterial extracts from *Aggregatibacter actinomycetemcomitans* in the production of IL-6 and IL-8 by human gingival fibroblasts**

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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 to aggressive forms of periodontitis. This microorganism express a cytolethal 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 anti-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 no 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 respiratory bacteria

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Objective: It is known that carriage of respiratory bacteria is a major factor in the transmission of the infection and in some cases the invasion 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. SPSS 15 was used for statistical analysis.
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 concentration 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

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TGF-β 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-β negatively regulates the inflammatory response by regulating lymphocyte influx to the airway and further modulating release of proinflammatory and anti-inflammatory cytokines. Eight-week-old BALB/c mice were intranasally instilled with influenza A virus (A/Udorn/317/72/H3N2), 4.1×10^3 PFU of virus in 50 µl of allantoic fluid or mock infected 50 µl of allantoic fluid. rTGF-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 perivascular spaces were extensively infiltrated by a mixture of inflammatory cells on 3rd, 5th and 7th days after influenza virus infection. An increase of INF-g 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

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Objectives: 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 immunoparalysis. In experimental animals, co-administration of PCT with bacterial lipopolysaccharide (LPS) increased lethality, while immunoneutralisation 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)

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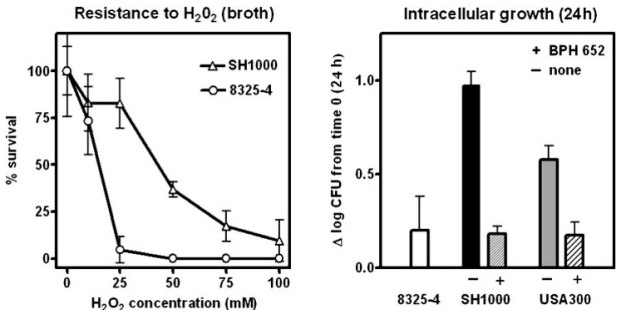
Background and Aims: *S. aureus* intracellular survival is critical for persistence of infection. rsbU expression stimulates the production of Staphyloxanthin (SFX), a yellow pigment that protects *S. aureus* against oxidant damage (Science [Wash.] 2008; 319:1391–1394). We have examined the role of rsbU and SFX in phagocytosis and growth of *S. aureus* in phagocytic cells.
Methods: Bacteria: strain 8325–4 (natural deletion in rsbU) and SH1000 (isogenic rsbU+ construct); USA300. Cells: THP-1 macrophages cultured and infected as previously described (AAC 2008; 52:2797–805). Impairment of SFX synthesis: BPH-652 (3-phenoxy-alpha-phosphono-benzenebutanesulfonic acid [dehydrosqualene 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.

P736 *Staphylococcus aureus* secretes two homologues Fc gamma receptor antagonists

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Objectives: Fc gamma receptors (FcgR) 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 FcgR have not been described yet. Our goal was to investigate whether *S. aureus* secretes any FcgR antagonists.
Methods: Culture supernatants of various clinical and laboratory *S. aureus* strains were screened for their ability to inhibit specific FcgR staining on phagocytes, analyzed by flow cytometry. An inhibitory protein was purified from one of the effective staphylococcal supernatants using dye-ligand affinity chromatography, gel filtration, and FcgR coated magnetic beads. Subsequently the purified protein was identified by mass spectrometry. To determine its FcgR modulating capabilities recombinant protein was generated. Direct binding of the inhibitor to the different FcgR subclasses, its ability to block FcgR-IgG interaction and its effect on FcgR mediated phagocytosis and intracellular killing were analysed using FACS and ELISA.
Results: *S. aureus* is able to evade FcgR mediated immunity by secreting two potent FcgR antagonists, FLIPr and its homologue FLIPr-like. Both proteins bind to FcgR and block FcgR-IgG interaction. They inhibit FcgR 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 FcgR mediated diseases, like allergy and autoimmunity.

P737 Biocide exposure activates sae promoter activity in *Staphylococcus aureus* strain Newman and increases cellular invasion

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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 cytometric 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.

P738 Phenotype-specific small non-protein-coding RNAs of *Staphylococcus aureus*

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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 Holo- and apo-transferrins interfere with adherence to abiotic surfaces and with adhesion/invasion to HeLa cells in staphylococci

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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 holo-transferrin (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 mg/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 leukocidin expression by *Staphylococcus aureus*

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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 each 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 where treated with either oxacillin or imipenem but not with cefotaxim, cefaclor or cefoxitin. Imipenem 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*.

P741 Sialic acid: a preventable signal for pneumococcal biofilm, colonisation and invasion of the host

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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 mucosae, 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.

P742 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*

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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

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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 (cylM, cylB, cylA, cylI, 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), 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 16S rDNA and rpoA.
Results: The expression level of the virulence-related genes analysed varied significantly considering all the conditions assayed ($P < 0.05$). Serum 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 metronidazole vaginal gel treatment and relapse of bacterial vaginosis

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Introduction: Bacterial vaginosis is the common cause of abnormal vaginal discharge among women of reproductive ages.
Objective: This is a randomised clinical trial study to compare the effect of vitamin C vaginal tablet with metronidazol gel in treatment of symptomatic bacterial Vaginosis.
Methods: After confirmed diagnosis of bacterial vaginosis to Amsel criteria (at least 3 out of the 4 characteristic symptoms discharge, fishy odour, vaginal pH $\geqslant$ 4.5, and presence >20% of clue cell), 60 non pregnant women of 15–45 years of reproductive ages enrolled in randomised clinical study. The women were randomly assigned to receive either 250 mg vaginal tablet vitamin C once daily for 6 days ($n = 30$) or vaginal gel 0.75% metronidazol 5 gr once daily for 5 days ($n = 30$). The two groups resulted comparable for demographics, history and baseline clinical picture. Participants were evaluated in two follow-up visits (after treatment and two week after treatment). Therapeutic success was defined as the presence of less than three of Amsels criteria.
Results: At the first follow-up visit, 23 (76.7%) of women in vitamin C group and 24 (80%) in metro gel group were cured ($P = 0.7$). At second follow-up visit two women (9.5%) in vitamin C group and one (5%) in metro gel group bacterial vaginosis was relapse ($P = 0.999$). Three women in vitamin C group and four in metro gel group were reported occasional burring and itching during product use.
Conclusion: Vitamin C vaginal tablet 250 mg has effectiveness as metronidazol vaginal gel for treatment of bacterial vaginosis.

Antibiotic use in hospitals and the community

P745 Non-compliance with guidelines for surgical antibiotic prophylaxis and the risk of surgical site infection: results from the INCISO Surveillance Network

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Objectives: Antibiotic prophylaxis reduces the incidence of surgical site infection (SSI) for many procedures. Nonetheless, these medications are not always given appropriately. The aim of this study was to describe practices of surgical antibiotic prophylaxis (SAP) and to determine whether the risk of SSI vary according to these practices.
Methods: Data were collected during a 7-year surveillance period (2001 to 2007) from the INCISO surveillance network based on volunteer participation of surgery wards in Northern France. Major aspects of SAP were evaluated including antibiotic agent choice, timing administration of first dose, total SAP duration and compliance to standard guidelines. The study focused on a few standard procedures in digestive, orthopaedic, gynaecological and cardiovascular surgery, for which a SAP is usually recommended. Univariate and multivariate logistic regression analyses were carried out to identify SAP-, patient-, and procedure-specific factors associated with SSI.
Results: Of 8029 surgical procedures which were assessed for compliance to guidelines, 7330 (91.3%) received SAP and 184 (2.6%) developed SSIs. Among patients receiving SAP, 83.3% received appropriate antibiotic agent and 82.6% had an optimal timing of administration. SAP was considered to be appropriate in 35.0%, unnecessarily prolonged in 45.2%, and shorter than recommended in 19.8% (e.g. when a single dose was administered instead of several for a longer surgical procedure). After adjustment for surgical speciality, NNIS risk index, age, and pre-operative hospital stay in the multivariate analysis, the risk of SSI increased significantly if SAP was shorter than recommended (OR = 1.8, 95% Confidence Interval: 1.2–2.8). Non significant relationships were observed between SSI and the other SAP parameters.
Conclusions: Inappropriate SAP duration was the most significant non-compliant practice associated with an increased SSI risk after some standard procedures. Information towards practitioners should be reinforced based on standard guidelines.

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

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Objectives: The aim of the study was to analyze the antimicrobial therapy administrated 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 administrated 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: Empiric 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.

P747 Efficacy of tigecycline versus ceftriaxone and metronidazole for complicated intra-abdominal infections: Americas study 400

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Background: Tigecycline (TGC) has demonstrated clinical efficacy and safety versus imipenem-cilastatin in previous clinical trials in subjects with complicated intra-abdominal infections. The current study was a multicentre, open label, randomised, comparative study of tigecycline versus ceftriaxone (CTX) plus metronidazole (MET) for the treatment of hospitalised subjects with complicated intra-abdominal infections conducted in 6 countries in North, Central and South America.
Methods: Subjects meeting the definition of complicated intra-abdominal infection (i.e. local or systemic infections secondary to a physical perforation in the gastrointestinal tract or via a necrotic gut wall into the peritoneal space, leading to abscess formation or peritonitis) and requiring surgery or a percutaneous drainage procedure in addition to intravenous antibiotics were randomised to receive TGC 100 mg loading dose followed by 50 mg q 12 hours or CTX 2 grams once daily, plus MET 1 gram to 2 grams daily, for a minimum of 4 days and not more than 14 days. Patients were stratified at randomisation for APACHE II scores $\leqslant 10$ or >10. Subjects were not allowed to receive oral therapy. The primary efficacy endpoint was the clinical response in the clinically evaluable (CE) population at the test of cure (TOC) assessment up to 21 days after the last dose of test article.
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

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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 b-lactamases (ESBL), commonly found in hospitals, has severely limited the number of antimicrobial agents available for use. Tigecycline is a first-in-class glycilcycline 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 carbapenens, 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 carbapenens can not be use.

P749 Macrolide use in intensive care units

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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 sulbactam. 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 systemic acting prokinetic drug e.g. to treat postoperative adynamic ileus in patients undergoing abdominal 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

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Objective: 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

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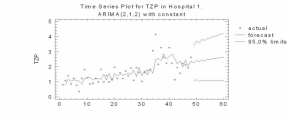
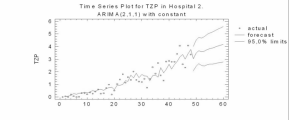
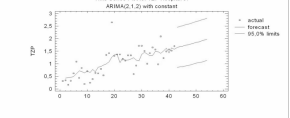
Objectives: Determine the relationship between the piperacillin-tazobactam (TZP) consumption and resistance of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to this antibiotic in three high complexity hospitals from Colombia.

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.

	Models for *K pneumoniae*		Models for *P aeruginosa*		Time Series Plot for TZP in DDD 100 bed-days. 2004 - 2007
Hospital 1	R2	0,12	R2	0,159	
	TZP (Order 1)	0,38	TZP (Order 2)	0,28	
	KPN (AR Order 3)	0,36	TZP (Order 9)	0,35	
			PAE (MA Order 1)	0,59	
			PAE (AR Order 5)	0,43	
Hospital 2	R2	0,12	R2	0,21	
	Constant	1,21	TZP (Order 1)	0,48	
	TZP (Order 3)	0,44	PAE (AR Order 1)	0,3	
	TZP (Order 14)	0,68			
	KPN (AR Order 2)	0,26			
Hospital 3	R2	0,1	R2	0,08	
	Constant	0,84	TZP (Order 5)	10,75	
	TZP (Order 6)	25,17	PAE (MA Order 2)	-0,33	
			PAE (AR Order 1)	0,36	
			PAE (AR Order 10)	0,44	

KPN: *K pneumoniae*. PAE: *P aeruginosa*. TZP: Tazobactam piperacillin.
R2: R-square. Order: Delay of the effect (months). Parameters of model (p<0.05).

P752 Surveillance of antimicrobial consumption patterns in high complexity hospitals in Colombia, 2002–2007

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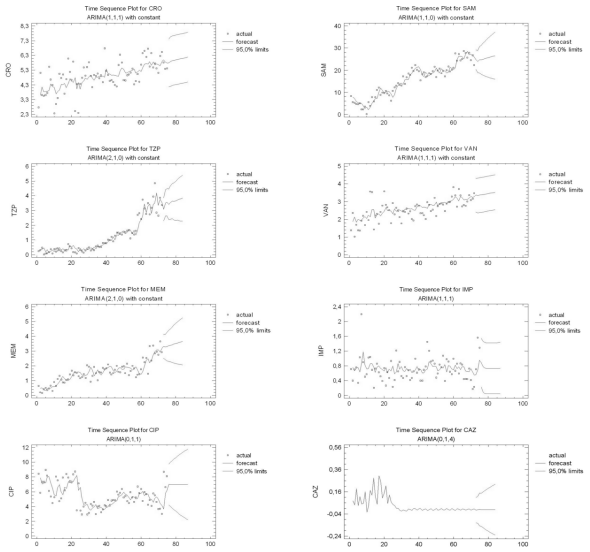
Objectives: Describe novel information about antimicrobial consumption patterns in a reference hospital wide network in Colombia

Methods: Surveillance of antimicrobial consumption prescribed in hospitals (intravenous indications) of ten wide used antibiotics in the country, from january 2002 to december 2007. The following antimicrobials were studied: amikacin, ampicillin/sulbactam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, clindamycin, imipenem, meropenem, oxacillin, piperacillin/tazobactam, vancomycin. Ten reference hospitals from five most important cities in the country including capital city reported antimicrobial consumption in milligrams from pharmacy registers, prescribed drugs were grouped as the number of defined daily doses (DDD) per 100 bed-days (World Health Organization). Information in DDD per 100 bed-days was monthly grouped. All descriptive analysis were performed, we describe temporal patterns using time series analysis of median consumption for each month (72 periods).

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.



CRO: Ceftriaxone. SAM: Ampicillin Sulbactam. TZP: Piperacillin. Tazobactam VAN: Vancomycin. MEM: Meropenem. IMP: Imipenem. CIP: Ciprofloxacin. CAZ: Cefatzidime.

Figure 1. 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 universitary 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 (beta/lactams time > MIC: penicillins >50%, cephalosporins >65%, carbapenems >40%; quinolones AUC/MIC > 100; aminoglucosides 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 universitary 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.

Antibiotic	N	% reaching PD target	% of dose reduction (range)	% of dose increase (range)
Ampicillin/sulbactam	120	93.3	71 (9–97)	52 (3–84)
Cephalotin	36	27.7	18 (3–28)	147 (7–404)
Oxacillin	16	86.6	79 (10–81)	2768 (66–5470)
Piperacillin/tazobactam	10	80	36 (19–62)	13 (2–24)
Ciprofloxacin	9	0	0	261 (50–513)
Ceftriaxone	8	100	70 (0–90)	0
Meropenem	3	100	60 (30–78)	0
Aminoglycosides	3	100	50 (22–70)	0

P754 Ten years of antibiotic consumption in ambulatory care: trends in prescribing practice and antibiotic resistance in Austria

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Objectives: 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 therapeutical 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 DIDs 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), amoxicillin/clavulanic acid (+61.9%), cefpodoxim (+31.6), azithromycin (+24.7) and decreased for erythromycin (−79.5%), trimethoprim (−56.1%), norfloxacin (−48.8%), doxycycline (−44.6), cefaclor (−35.1%), penicillin [(−34.0%), (pen)] amoxicillin (−22.5), minocycline (−21.9%) and clarithromycin (−9.9%). Since 2001, an increase in the percentage of resistant invasive *E. coli* isolates for aminopenicillins (from 35%-53%), fluoroquinolones (from 7%-25.5%) and 3rd generation cephalosporins (from 0%-8.8%) was observed. The percentage of pneumococcal isolates (spn) 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 (amoxicillin/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

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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 (TBP) 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 TBP-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 (dis)advantages of using AB guidelines?); SN (Are there any individuals or groups that would (dis)approve 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 a paediatric department among 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

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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)

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=±100/year). Underdosing 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 has been a major factor in reducing antibiotic pressure and total cost in the hospital. Continuous registration and antibiotic stewardship by AMT 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

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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

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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 sent in 24% 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.

Results: Ninety-five medical patients, (age range: 19–92 years) were prescribed 126 antimicrobials in the ED in September 2008. The most commonly prescribed antibiotics were co-amoxiclav (56), clarithromycin (24) and piperacillin-tazobactam (13). 63.5% (80/126) of prescriptions were appropriate. The referral of microbiological investigations also increased to 81.1% (77/95 patients). Attendance at the weekly microbiology sessions has been good and feedback positive.
Conclusions: Antimicrobial prescribing in the ED improved following the introduction of education sessions and the 'quick reference' antimicrobial guide. The weekly five-minute antimicrobial information sessions were particularly successful in targeting one 'key message', however it was important to also target the ED specifically with education sessions due to staff rotas making attendance at medical rounds difficult. As doctors in the ED continue to change, educational sessions are ongoing, the antimicrobial guide will be reviewed in July 2009 and compliance re-audited in September 2009.

P759 A one-day prevalence study: the evaluation of antibiotic use and cost in a training hospital

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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 diseaes 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

	Medical wards		Surgical wards		Total	
	n	%	n	%	n	%
Ampicillin-sulbactam	57	19.3	58	16.0	115	17.5
Cefazolin	6	2.0	122	33.7	128	19.4
Ceftriaxone and cefotaxime	41	13.9	44	12.1	85	12.9
Ceftazidime	5	1.7	–		5	0.7
Cefoperazone-sulbactam	22	7.4	4	1.1	26	3.9
Piperacillin-tazobactam	46	15.6	28	7.7	74	11.2
Imipenem/cilastatin and meropenem	41	13.9	14	3.8	55	8.3
Teicoplanin and vancomycin	25	8.4	15	4.1	40	6.0
Linezolid	4	1.3	2	0.5	6	0.9
Gentamicin and amikacin	8	2.7	23	6.3	31	4.7
Ciprofloxacin and levofloxacin	11	3.7	4	1.1	15	2.2
Metronidazole	13	4.4	44	12.1	57	8.6
Clarithromycin	5	1.7	–		5	0.7
Other antibiotics	11	3.7	4	1.1	15	2.2
Total	295	100.0	362	100.0	657	100.0

Conclusion: This study showed that the antibiotic usage rate was 40.4, inapproppriate 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.

P760 Antibiotic consumption in Lithuanian general and nursing hospitals and influencing factors

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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 ABC Calc 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 χ^2 or Fisher's exact test for proportions.
Results: There was huge variation of antibiotic consumption (ranging from 11.5 tol 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.

P761 Sustained favourable impact of an antibiotic management programme on the prescription of fluoroquinolones in a tertiary hospital after 4 years

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Objectives: The increase of bacterial resistance and of fluoroquinolones (FQ) consumption led us to set up an action plan in order to improve the use of FQ.
Methods: The impact of the programme was evaluated by comparing results of on a given day audits on FQ prescriptions, done in all wards of our institution (medicine, surgery and ICU) in 12/2005 (ie before implementation of the action), in 01/2007 and in 12/2008. Global antibiotics and FQ consumption in our hospital were also compared throughout the intervention. Main points of the action led by the antimicrobial stewardship programme team were as follows: feedback to the prescribers of the first and second one day audits, redaction and diffusion to all prescribers of a pocket-size leaflet of recommendations on FQ utilisation, direct daily educational interaction with the prescribers for antibiotic prescriptions, with emphasis on FQ use.

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

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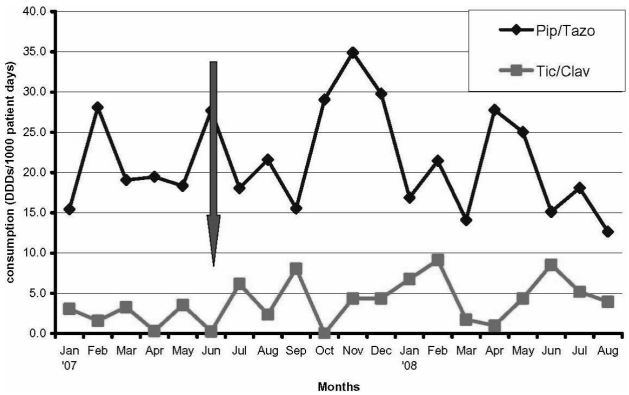
Background and Objectives: According to an antibiotic restriction policy implemented in Hellenic hospitals since late 80 s, all potent antibiotics (3rd and 4th generation cephalosporins, aztreonam, carbapenems, quinolones and glycopeptides and more recently linezolid, daptomycin and tigecycline) are 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: P/t consumption was 15.4, 28.1, 19.1, 19.5, 18.3, 26.8 DDDs per 1000 patient days for the months January 2007 to June 2007 and 18.1, 21.6, 15.6, 29.1, 34.9, 29.8, 16.8, 21.5, 14.1, 27.8, 25.0, 15.1, 18.1, 12.7 for the months July 2007 to August 2008, respectively, and no statistical trend was found. The values for t/c consumption were 3.1, 1.6, 3.3, 0.3, 3.5, 0.3 (January to June 2007) and 6.1, 2.4, 8.1, 0, 4.4, 4.1, 6.8, 9.1, 1.7, 1.0, 4.4, 8.6, 5.2, 3.9 for the 14 months after restriction and again no trend was found. Furthermore, the mean per month consumption for p/t was 20.1 and 21.6 DDDs per 1000 patient days before and after restriction implementation respectively, and that of t/c 2.4 and 4.7 DDDs per 1000 patient days respectively.
Conclusions: The addition of t/c and p/t to the antibiotic restriction list failed to reduce their consumption in our hospital during the study period. Besides it is worth noting that, almost two decades after the introduction of the above mentioned restriction policy in the Hellenic hospitals, no formal audit has ever been performed. Perhaps it is time for us to revise our antibiotic policy.

P763 Antibiotic therapy in critically ill patients: rates of empiric appropriate or adequate therapy and subsequent adaptation of therapy in daily practice

D. Vogelaers, D. De Bels, F. Forêt, S. Cran, E. Gilbert, K. Schoonheydt, S. Blot for the Anthicus Study Investigators*

Objectives: To investigate rates of empiric appropriate or adequate antibiotic therapy and subsequent adaptation of therapy in ICU patients with severe nosocomial infections.
Methods: A prospective, observational multicentre study was performed (Januari to December 2007) in which ICU patients with nosocomial infection were included. Appropriate therapy was defined as in vitro susceptibility of the causative pathogen and clinical response to the antibiotic agent. In non-microbiologically documented infections, empiric therapy was considered adequate in case of favourable clinical response within 5 days of therapy.

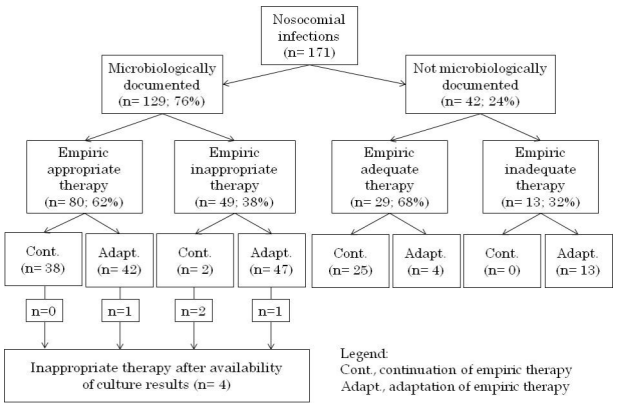


Figure: Rates of empiric appropriate or adequate therapy in ICU patients with nosocomial infections.

Results: 171 patients from 28 centres were included (62% male; mean age 67 years, interquartile range (IQR) 55–76; mean APACHE II score 20, IQR 15–24). Patients had pneumonia (n = 131, 68 ventilator-associated), intra-abdominal infection (n = 23), bacteraemia (n = 16) or sinusitis (n = 1). 115 infections were ICU-acquired (67%). In 130 infections a causative pathogen was isolated (76%). Gram-negative aetiology was most prevalent (n = 92; 75%) with *Pseudomonas aeruginosa* (n = 28), *Escherichia coli* (n = 26) and *Enterobacter aerogenes* (n = 21) being most common. In 51% of infections (n = 64) multidrug resistant (MDR) bacteria were involved (ESBL, MDR nonfermenting Gram-negatives, or MRSA). Appropriateness/adequacy of empiric therapy and subsequent rates of adaptation are shown in the figure. In 106 patients empiric therapy was adapted, in 60 cases following initial inappropriate/inadequate therapy, in 46 patients in order to fine tune empiric therapy. Rates of empiric appropriate/adequate therapy were highest with use of meropenem, either in mono (89%) or combination therapy (90%). These rates didn't change when only patients with particular risk factors for MDR involvement (prior hospitalisation or antibiotic exposure) were taken into account.

Combinations mainly based on piperacillin-tazobactam, amoxicillin-clavulanate, cephalosporins, quinolones or glycopeptides never reached rates of appropriate/adequate therapy in excess of 60%. Surprisingly, these rates did not increase considerably for patients without MDR risk factors only.
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.

P764 *Staphylococcus aureus* bloodstream infection management indicators as quality indicators for hospital antibiotic stewardship: feasibility study by the ABS International Quality Indicators (ABS QI) team

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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 betalactam 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.

P765 Adequacy of antimicrobial treatment and outcome of *Staphylococcus aureus* bacteraemia in nine European countries

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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 (methicillin-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 methicillin 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 patient source records.
Conclusion: In a representative sample of 60 hospitals in 9 West-European hospitals 28.4% of patients with SAB received inadequate empirical therapy, which was strongly associated with infection caused by MRSA. In this study inadequate treatment or SAB due to MRSA (compared to MSSA) were not associated with higher 30-day mortality.

Table 1. Univariate and multivariate analysis for the outcome of 30-day all cause mortality

Variable	Alive	Death (all causes)	Univariate		Multivariate	
	n = 254	n = 80	OR (95% CI)	p-value	OR (95% CI)	p-value
Teaching (vs non-teaching) hospital (%)	197 (77.6)	55 (68.8)	0.64 (0.37–1.11)	0.11		
Age, median (IQR)	66.0 (54–76.0)	74.5 (62.5–83)	1.04 (1.02–1.05)	<0.001	1.06 (1.03–1.10)	0.001
Male (vs female) (%)	170 (66.9)	54 (67.5)	1.03 (0.60–1.75)	0.93		
Adapted Charlson comorbidity score, median (IQR)	3.0 (0–5)	4.0 (2–6)	1.18 (1.07–1.30)	0.001	2.09 (1.21–3.63)	0.008
Immunocompromised (vs non-immunocompromised) (%)	32 (12.6)	8 (10.0)	0.77 (0.34–1.75)	0.53		
Secondary (vs primary) bacteraemia (%)	94 (37.0)	28 (35.0)	0.92 (0.54–1.55)	0.75		
Length of stay before onset of SAB, median (IQR)	2.0 (0–10)	2.0 (0–9.5)	1.00 (0.98–1.01)	0.67		
Severe sepsis or septic shock (vs sepsis) at onset of SAB (%)	77 (30.3)	49 (61.3)	3.63 (2.15–6.13)	<0.001	2.68 (1.52–4.75)	0.001
ICU (vs non-ICU) at onset of SAB (%)	44 (17.3)	29 (36.3)	2.71 (1.55–4.75)	<0.001	2.89 (1.48–5.64)	0.002
Inadequate (vs adequate) initial treatment (%)	75 (29.5)	20 (21.1)	0.80 (0.45–1.41)	0.48	0.69 (0.36–1.32)	0.26
MRSA (vs MSSA) (%)	57 (22.4)	20 (25.0)	1.15 (0.64–2.07)	0.64	0.98 (0.50–1.94)	0.95
Age Adapted Charlson comorbidity score			1.002 (1.001–1.004)	<0.001	0.99 (0.98–0.999)	0.03

P766 Clinical experience with daptomycin in Europe

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Objective: To describe the clinical experience with daptomycin (DAP) in Europe since marketing authorisation in January 2006.
Methods: The European Cubicin® 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, bacteraemia, 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%), bacteraemia (22%), endocarditis (12%), uSSTI (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%.

P767 Patient factors impacting the transition from inpatient to outpatient daptomycin treatment

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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 Cubicin 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, bacteraemia 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%), bacteraemia (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/mm^3 (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 contributors 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 as an 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.

P768 Outpatient parenteral teicoplanin treatment in staphylococcal prosthesis infections

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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 retrosepectively 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 valuations 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.

P769 Impact of nasal methicillin-resistant *Staphylococcus aureus* surveillance culture results on subsequent antibiotic prescribing patterns

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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.

P770 Cost-effectiveness of daptomycin in hospitalised patients with cSSTI caused by Gram-positive organisms

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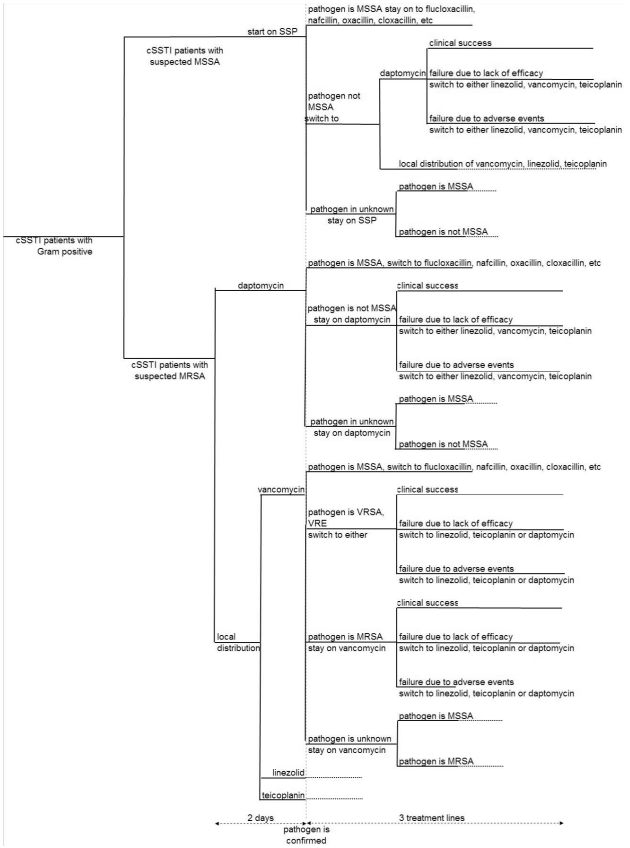
Objectives: Mortality associated with complicated skin and soft tissue infections (cSSTIs) due to MRSA infections has been increasing in the UK creating significant economic and humanistic burden. Daptomycin is a new, cyclic lipopeptide antibiotic for the treatment of cSSTI caused by Gram positive bacteria. 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.



P771 Utility of unique procalcitonin dosage in patients with acute exacerbation of chronic obstructive pulmonary disease in emergency wards

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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 = 12.5; 95% CI 4.86–32.20; P < 0.0005). Inadequacy of treatment was 9/39 in standard conditions and should have been 32/39 with the PCT-guided model (OR = 15.2; 95% CI 5.04–46.0; P = 0.0059).
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

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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 Tarification 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 antimycotics for systemic use (D01BA, J01, J02&P01AB), and tuberculostatics (J04A).

Table 1. Overview of DDD/1000 bed days in participating hospitals

Class	ATC	2006 (N = 24)		2007 (N = 46)	
		mean	range	mean	range
Beta-lactam antibacterials, penicillins	J01C	235	168–375	253	85–380
Other beta-lactam antibacterials	J01D	111	48–175	116	14–230
Quinolone antibacterials	J01M	65	8–102	69	7–115
Other antibacterials[a]	J01X	36	13–61	38	10–81
Antimycotics for systemic use	J02A	26	0–72	35	0–111
Macrolides, lincosemides and streptogramins	J01F	24	9–38	26	5–64
Aminoglycoside antibacterials	J01G	15	3–38	15	1–38
Drugs for treatment of tuberculosis	J04A	12	0–132[b]	11	0–110[b]
Sulfonamides and trimethoprim	J01E	9	1–22	9	1–29
Intestinal antiinfectives	A07A	4	0–21	3	0–14
Agents against amoebiasis and other protozoal diseases	P01A	3	0–7	4	0–10
Tetracyclines	J01A	3	1–13	4	1–44
Antifungals for systemic use	D01B	1	0–6	1	0–6
Amphenicols	J01B	0	0–1	0	0–2
Total		545	344–827	583	225–929

[a] Glycopeptide antibacterials, polymyxins, steroid antibacterials, imidazole derivatives, nitrofuran derivates and others.
[b] Hospital specialised in HIV.

Results: A total of 24 and 46 of the intended 37 and 61 hospitals participated in 2007 and 2008 (data for 2006 and 2007), respectively. 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 (J01C) and antimycotics (J02A) 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.

P773 Improving patient safety in Europe. Standards and performance indicators in healthcare-associated infection and antimicrobial stewardship

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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.

P774 Linezolid for patients with neutropenia: can bacteriostatic agents be used in this patient population?

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Objective: A long held doctrine is that bactericidal 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?

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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 (DID) (ATC-DDD 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/10000 inh, physicians quantity (v24), physicians quantity/10000 inh, number of inh/physician, nurses quantity, nurses quantity/10000 inh, number of inh/nurse, morbidity, some infectious and parasitic diseases morbidity, retailment turnover, retailment 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.

Region	AC (DID)		
	2004	2005	2006
Voronezhskaya oblast	11.08	12.9	12.23
Krasnodarskii kray	8.62	7.08	6.08
Krasnoyarskii kray	7.16	9.59	9.62
Nizhegorodskaya oblast	8.09	8.59	8.37
Novosibirskaya oblast	10.04	12.24	12.43
Omskaya oblast	6.40	7.29	7.26
Bashkortostan	9.74	11.06	11.88
Tatarstan	8.92	9.92	10.45
Rostovskaya oblast	4.94	7.12	6.93
Samarskaya oblast	8.55	7.76	8.38
Sverdlovskaya oblast	7.94	7.38	7.19

After multiple regression analysis carrying out we defined dependence model: DID = 93.905 – 0.15*v2 – 3.8311*v9 – 9.3132*ln(v13) – 11.1692*ln(v14) + 0.0154*v15— 0.3549*v17 – 0.6319*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

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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 dispensed during the annual meetings of paediatricians, 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 phycisians 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 pneumoniae (40%) and COPD (30%) and overused in acute diarrhoea (33–40%), patients with indwelling urinary catheters (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

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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

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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.

Molecular bacteriology – part 1

P779 Testing for *Clostridium difficile* infection

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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), Xpert™ *C. difficile* PCR (Cepheid), Gene Ohm™ PCR (BD Diagnostics) and C. DIFF QUIK CHEK COMPLETE™ (TechLab). The latter of these tests independently detects both glutamate dehydrogenase antigen (GDH) and *C. difficile* toxin (CDT). 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 CHEK 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 CHEK COMPLETE™ CDT component and VIDAS EIA (see table).

Test under evaluation	Gold standard used for comparison	Sens (%)	Spec (%)	PPV (%)	NPV (%)
C. DIFF QUIK CHEK COMPLETE™					
GDH antigen	*C. difficile* culture	100	98.4	90.9	100
C. difficile toxin	Stool cytotoxicity assay	71.4	100	100	97.0
VIDAS® *Clostridium difficile* A&B	Stool cytotoxicity assay	71.4	100	100	97.0
Xpert™ *C. difficile* PCR	Stool cytotoxicity assay	100	96.9	77.8	100
Gene Ohm™ PCR	Stool cytotoxicity assay	100	98.4	87.5	100

Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value.
GDH = glutamate dehydrogenase.

Conclusions: The C. DIFF QUIK CHEK COMPLETE™ GDH component and both of the PCR tests studied all have very high sensitivities and can therefore be relied upon for the exclusion of CDI. Since the PCR tests detect the presence of genetic material encoding for CDT, false positive results due to non-toxigenic organisms are eliminated. Furthermore, the Xpert™ *C. difficile* test can indicate the presence of an 027 ribotype. A small number of samples were positive by PCR but had negative stool cytotoxicity assay results, possibly reflecting faecal carriage of *C. difficile* organisms with repressed toxin production. The significance of this observation warrants further study.

P780 Determining hypervirulent markers for *Clostridium difficile* by array comparative genomic hybridisation

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Objectives: The aim of the study 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 (WTSI, 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 027 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 *C. difficile* 630.

P781 Toxin genes (tdcA, tdcB, cdtA and cdtB) of *Clostridium difficile* strains isolated from patients with *C. difficile*-associated diarrhoea in Turkey

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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 cultivation, 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 antigenetic diversity among the cytotoxins TcdB and use of novel monoclonal antibodies to diagnose *Clostridium difficile* ribotype strains

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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 lateron 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 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 Kuijper (Leiden University Medical Centre)

P783 Evaluation of a combined toxinA/B PCR for the detection of *Clostridium difficile* in a general hospital

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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 −80ºC and screened retrospectively with PCR. DNA extraction was performed with the Xtractor Gene (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

	CTA−[a] (113)	CTA+/culture+ (15)	CTA+/culture− (15)
PCR	110 negative, 3 positive[b]	3 negative, 12 positive	15 negative

[a]Culture not performed. [b]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

	Negatives (125)	Positives (16)
CTA	110 negative (specificity 88%)	15 positive (sensitivity 93.8%)
PCR	125 negative (specificity 100%)	13 positive (sensitivity 81.3%)

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

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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 cytoxicity, 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 cytoxicity und 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*

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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 diarroeal 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, toxigenic 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 5800 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

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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 toxinotype ($P > 0.05$). 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.

P787 Preliminary molecular evaluation of the toxigenicity of *Clostridium difficile* strains isolated from dogs in the area of Parma (Italy)

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Objectives: *Clostridium difficile* has been associated with canine acute and chronic large and small bowel diarrhoea, as well with 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.

P788 Comparison of a multiplex real-time PCR and the cell cytotoxicity neutralisation assay for the diagnosis of *Clostridium difficile* infections

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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 (tcd B), binary toxin (ctd A/B), and the tcdC deletion nt 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.

P789 Real-time PCR assays for the simultaneous detection of gyrA and gyrB mutations in *Clostridium difficile* isolates

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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 Asn or 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 Tm of 52ºC, compared to a Tm of 59ºC of the wild type. Strains with the substitution Asp to Asn in GyrB426 showed a Tm of 53ºC, strains with the substitution Asp to Val in GyrB426 a Tm of 54ºC and the wild type and the strains with a change in GyrB427 a Tm of 59ºC. A gyrB allele characterised by 3 silent mutations not involved in resistance showed a Tm of 50ºC. 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.

P790 **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)**

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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 vanRSHAXYZ (11 kb) and vanRSYWHBX (6Kb) Long-PCR and PCR products were digested with ClaI y 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 or in the stability of these resistant determinants among endemic STs.

P791 **Vancomycin-resistant *Enterococcus* in Canada: results from the Canadian Nosocomial Infection Surveillance Program, 1999–2007**

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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 $\geqslant$8 mg/mL is isolated from a clinical or screening specimen. Infection was defined as the presence of an illness that met standard infection surveillance definitions. Colonisation was defined as the presence of VRE in surgical wounds, urine, stool (rectum), or other body sites in an individual not manifesting clinical signs and/or symptoms. To be defined as health-care associated, there had to be no evidence that the organism was likely present at the time of admission.
Results: From 1999 to 2007, the rate of VRE increased from 0.37 to 2.48 cases per 1,000 admission s (do you mean, admissions). The increase in the rate was due primarily to an increase in VRE colonisation, from 0.34 to 2.74 cases per 1,000 admissions ($p < 0.0.001$). The rate of VRE infection increased from 0.02 to 0.08 cases per 1,000 admissions. The overall incidence of VRE increased from 1.20 per 1,000 admissions in 2006 to 2.48 per 1000 admissions in 2007 ($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%.

P792 **A probiotic approach to combat multiresistant enterococci: a cross-over clinical trial on the effect of probiotics on nosocomial spread of ampicillin-resistant *Enterococcus faecium***

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Background: Colonisation and infection rates with ampicillin-resistant *Enterococcus faecium* (ARE) have increased in our hospital. We assessed whether oral probiotics (microbial food supplements that improve intestinal colonisation resistance) decreased the risk of acquisition of intestinal ARE-colonisation in hospitalised patients (pts).
Methods: In a prospective cohort study with cross-over design all eligible pts (length of stay [LOS] >48 h) on two wards where ARE-colonisation is endemic (gastro/nephrology [ward1] and geriatrics [ward2]), were offered a multispecies probiotic product (5 gram;109 cfu/g) 2×/day until discharge (4.5 months, probiotic period) or not (4.5 months, control period). During the entire study, all pts were screened for perianal ARE-carriage <48 h after admission, 2×/week and <48 h before discharge. Data were analysed with a Cox proportional hazards model with time-dependent covariates. Colonisation pressure (daily proportion of pts colonised with ARE), antibiotics (AB) with intestinal activity (subdivided in 2 classes according to activity against ARE), a post-antibiotic effect of 3 days (assuming the intestinal flora needs time to restore after stopping AB), treatment in isolation, age and medical specialty were analysed as potential confounders. AB active against ARE included piperacillin(/tazobactam) and oral vancomycin.
Results: During the study (267 days on ward1 and 296 days on ward2) 852 pts had a LOS > 48 h. 563 (66%) were screened on admission, of which 127 (23%) were colonised with ARE. In all, 1987 cultures were obtained and, on average, daily colonisation status was known for 69% of the pts. Of all pts at risk for ARE-acquisition (n = 436), 110 (25%) used probiotics during (part of) their stay and 92 (21%) acquired ARE-colonisation: 28 (25%) of the 110 that used probiotics and 64 (20%) of 326 control pts. After adjustment for colonisation pressure (hazard ratio (HR) 1.76 [95% CI 0.3–10.3]), AB inactive against ARE (HR=4.61 [95% CI 2.8–7.6]) and the post-antibiotic effect (HR= 5.25 [95% CI: 1.9–14.3]), the HR for probiotics was 0.53 (95% CI: 0.2–1.8). There was an interaction between probiotics and AB inactive against ARE: the combined use was associated with the highest risk of ARE-acquisition (HR=8.31 [95% CI 4.2–16.5]).
Conclusion: In the absence of antibiotic use, probiotics tend to have a protective effect on ARE-acquisition in hospitalised pts. In pts using AB inactive against ARE, probiotics increase the hazard of acquisition from 4.61 to 8.31.

P793 Molecular epidemiology of *Staphylococcus aureus* among asymptomatic carriers from Saxony

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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 neurosurgical 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 egc comprising of seg, sei, sem, sen, seo, and seu 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 (9.0%).
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 & Ehricht, 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

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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 conformed case on the same unit.
Results: See table 1.

Table 1

MRSA results	Culture positive	Culture negative	Total
PCR positive	8	4	12
PCR negative	1	190	191
Total	9	194	203

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*

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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:orfX 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.

Conventional culture identification (n=2500)	MRSA by PCR	Non MRSA by PCR				
	Scc + mecA + Sa442 +	*Scc - mecA + Sa442 +*	*Scc - mecA - Sa442 +*	*Scc + mecA - Sa442 +*	*Scc - mecA + Sa442 -*	*Scc - mecA - Sa442 -*
MRSA (n=1299)	1241	57	----	1	----	----
MSSA (n=760)	3	9	649	46	----	53
Others (n=441)	---	----	----	----	261	180

Sensitivity = 95.6% (1241/1299)
Specificity = 99.8% (1198/1201)
PoPV = 99.8% (1241/1244)
NePV = 95.4% (1198/1256)

Calculated values	
TP 1241	FP 3
FN 58	TN 1198

Results and Diagnostic values for MSSA detection.

Conventional culture identification (n=2500)	MSSA by PCR		Non MSSA by PCR			
	Scc - mecA - Sa442 +	*Scc + mecA - Sa442 +*	*Scc + mecA + Sa442 +*	*Scc - mecA + Sa442 +*	*Scc - mecA + Sa442 -*	*Scc - mecA - Sa442 -*
MRSA (n=1299)	----	1	1241	57	----	----
MSSA (n=760)	649	46	3	9	----	53
Others (n=441)	----	----	----	----	261	180

Sensitivity = 91.5% (695/760)
Specificity = 99.9% (1739/1740)
PoPV = 99.9% (695/696)
NePV = 96.4% (1739/1804)

Calculated values	
TP 695	FP 1
FN 65	TN 1739

Results: Validation with 150 known staphylococcal strains reviled 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 redaction 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 staphylococci 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*

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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 NdeI and XhoI 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 NdeI and XhoI, 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

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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 (Copan) 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 untill 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 35ºC. 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

	eSwab® culture +	eSwab® culture –	Total
GeneXpert® +	28	5	33
GeneXpert® –	1	125	126
Total	29	130	159

P798 Sequence-based characterisation of the agrD gene and the hld gene in *Staphylococcus epidermidis* isolated from prosthetic joint infections

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Objectives: *Staphylococcus epidermidis* is a commensal that comprises a substantial part of the normal human skin flora. Nevertheless, this bacterium has emerged as the most important pathogen in infections related to implanted foreign body materials, especially prosthetic joint infections (PJIs).
A 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 PJIs, 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 PJIs 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 PJIs 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 PJI (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 commensal isolates 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 PJIs 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

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Objectives: The high level of *Helicobacter pylori* infection in Poland (about 60%) may constitute a risk factor of still high morbidity rate on gastroduodenal 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

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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 five predicted genes, 4899 bp in total length, presenting high homology to genes involved in the synthesis of sialylated lipopolysaccharade outer core in *Campylobacter jejuni* (neuB, neuC, neuA and two copies of cstII) 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 cytosine and 15 of guanine were detected upstream and downstream of the first cstII 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 cstII 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* lipopolysaccharade 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

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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 (fingi) 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 (omeprasole, amoxicillin, claritromicin) 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 pathogenecity island (PAI) of HP (ureC, cagA, cagC, cagA, cagH) and genes of CA (sap2 – secreted aspartic proteinases, hwp1 – hyphal wall protein 1, alp7 – agglutinin like protein 7) were studied.
Results: It was determinated, that at all DU patients carried both CA and HP before treatment. The HP genes encoding for PAI were determinated 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 determinated in 100% of patients, gene hwp1 – in 51.9%. After the anti-HP-therapy the percentage of determinating 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 Comparasion of Genotype® HelicoDR with real-time PCR to identify clarithromycin resitance in *Helicobacter pylori*

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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: 49 biopsies for *H. pylori* were obtained from patients with gastric symptoms, received at the Department of Microbiology from December 2007 to May 2008. Standard microbiological procedures were used for *H. pylori* culture. Clarithromycin resistance was determined by E-test. DNA extraction was carried out by the NucliSens easyMAG platform with the NucliSens magnetic extraction reagents (bioMérieux) according to the manufacturer instructions. MutaREAL® *Helicobacter pylori* Real Time PCR Kit and Genotype® HelicoDR was used to clarithromycin resistance in *H. pylori* following manufacturer recommendations.
Results: All biopsies showed a positive result with the MutaREAL® kit and Gentotype® HelicoDR. 24 out of 49 showed clarithromycin resitance by E-test. The Real time PCR detected clarithromycin resistance in 21 cases with a sensitivity and especificity 87.5% and 92%, respectively, compared with phenotypical methods. The Genotype® HelicoDR detected resistance in 27 cases: 20 biopsies had one point of mutation, 2 biopsies had two points of mutation and 5 biopsies showed hetero-resistance with a wild type and mutated strain. The sensitivity and specificity were 100% and 96%, respectively. The mutation A2143G was detected in all specimens, and the mutation A2142C in two biopsies with two points of mutation.
Conclusions: MutaREAL® Kit and Genotype®HelicoDR were able to detect clarithromycin susceptibility with high sensitivity and specificity and are quicker than culture. Genotype®HelicoDR had better sensitivity and specificity and it was possible to detect the type of mutation and hetero-resitance strains. Real Time PCR is suitable to be done in 1 hour after DNA extraction.

P803 Development of a new test, GenoType® HelicoDR, for molecular detection of antibiotic resistance in *Helicobacter pylori*

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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.

P804 Analysis of 3′ end variable region of cagA in *Helicobacter pylori* isolated from Iranian dyspeptic patients

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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.

P805 Frequency of vacA, cagA and babA2 virulence markers in *Helicobacter pylori* strains isolated from Mexican patients with chronic gastritis

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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 strains examined possessed the vacA s1, m1 (40.7%), s1, m2 (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.

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Antimicrobial susceptibility testing and resistance detection

P806 EUCAST breakpoints in automated susceptibility testing of Gram-negative bacteria – BD Phoenix validated

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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), cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ), cefuroxime (CXM), 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.

Organism group	CA first test		CA final test		Total tests
	n	%	n	%	(n)
P. aeruginosa	305	94.1	314	96.9	324
ESBL	443	92.3	463	96.5	480
Esch. coli	937	97.6	952	99.2	960
K. pneumoniae	652	98.8	660	100.0	660
K. oxytoca	176	97.8	180	100.0	180
P. mirabilis	321	97.3	328	99.4	330
M. morganii	176	97.8	179	99.4	180
E. cloacae	172	95.6	177	98.3	180
E. aerogenes	147	98.0	149	99.3	150
Total	3329	96.7	3402	98.8	3444

P807 EUCAST breakpoints in automated susceptibility testing of Gram-positive bacteria – BD Phoenix validated

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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.

Results: The results are summarised in the table. Overall category agreement was 99.4% for 2540 tests. Of note, all MRSA and MSSA strains were correctly identified as such with both cefoxitin and oxacillin. Among the enterococci, 12 VRE strains were correctly designated, as were 11 strains displaying gentamicin HLR. Inducible MLSB resistance was present in 8 strains of staphylococci, reported clindamycin R by disc testing and clindamycin S by PHX. However, PHX advises a test for inducible resistance, in line with EUCAST Expert Rule 11.3.

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.

Organism group	CA first test		CA final test		Total tests
	n	%	n	%	(n)
MRSA	414	98.6	418	99.5	420
MSSA	888	99.1	893	99.7	896
CoNS	353	98.1	353	98.1	360
S. lugdunensis	108	100.0	108	100.0	108
Str. pneumoniae	256	99.2	258	100.0	258
Str. pyogenes	244	99.6	244	99.6	245
Str. agalactiae	244	99.5	244	99.5	245
E. faecalis	141	100.0	141	100.0	141
E. faecium	109	97.3	109	97.3	112
Total	2513	98.9	2524	99.4	2540

P808 A new European standard method for disc diffusion antimicrobial susceptibility testing on Mueller–Hinton agar: a preliminary report

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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 β-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% CO_2 for 18±2 h. The inoculum suspension was 1 to 2 $\times 10^8$ 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 ± <3 mm). All inhibition zone diameters were within the CLSI ranges for organisms where comparison could be made. Compared to the corresponding CLSI target values for *E. coli* ATCC 25922, mean values of inhibition zone sizes were ⩽ 1 mm and ⩽2 mm for 21 and 28 of 29 antimicrobials, respectively. For *S. aureus* ATCC 25923, the corresponding figures were 27 and 30 of 31, respectively.
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 β-NAD/L can be used for non-fastidious and fastidious organisms, respectively.

P809 Comparison of results from testing sensitivity to amoxicillin/clavulanate with CLSI versus EUCAST interpretation

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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 2/1, 4/2, 8/4, and 16/8. The concentration of EUCAST combination were 1/2, 2/2, 4/2, 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.

	E. coli			*Klebsiella pneumoniae*		
	CLSI	EUCAST	DIN	CLSI	EUCAST	DIN
% Resistant	6.7	39.8	39.8	9.5	20.9	20.9
% Sensitive	77. 2		5.00	85.1		58.4

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.

P810 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

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Objective: 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 system (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, haemin, 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.

P811 Comparative evaluation of four commercial methods for antimicrobial susceptibility testing of a collection of 96 *Aeromonas* clinical isolates

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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,

bioMérieux, France), NC47 panel (C) (Walk/Away, Siemens, USA) and disk diffusion method (D) (Biorad, USA) was carried out according to the CA-SFM guidelines on 96 *Aeromonas* clinical strains. Testing was performed after control of each system. Results were compared to MIC-based categorisation determined with the agar dilution method (MIC-agar), used as gold standard. Antimicrobial drugs tested were: amoxicillin (AMX), co-amoxiclav (AMC), ticarcillin (TIC), ticarcillin+clavulanic acid (TCC), piperacillin (PIP), cefalothin (CFT), cefotaxim (CTX), cefepim (FEP), imipenem (IPM), nalidixic acid (NAL), ofloxacin (OFX), ciprofloxacin (CIP), cotrimoxazole (SXT), gentamicin (GEN), tobramicin (TOB), amikacin (AMK). Very major error (VME) and major error (ME) rates were determined only when denominators (numbers of true-R and of true-S, respectively) were $\geqslant$ 20.

Results: Categorical agreement levels were 82–98%, 78–98%, 82–95%, 79–100% with A, B, C, D, respectively for all antibiotics except for AMC, TCC and TIC, for which categorical agreement levels were lower: 42–75%, 6–59%, 20–64%, 4–73% with A, B, C, D, respectively. Concerning discrepancies, VME rates were acceptable ($\leqslant$1.5%, according to the CLSI guidelines), except for: AMX (C: 3%); AMC (A: 11%; B: 48%; C: 3%; D: 13%;); TIC (A: 8%; B: 15%; C: 15%); TCC (A: 10%; B: 52%; C: 42%; D: 10%); CFT (A: 1.7%; B: 5%; C: 8%; D: 5%). ME rates were acceptable ($\leqslant$3%, CLSI guidelines), except for: TIC (A: 28%; D: 24%), TCC (A: 33%), PIP (A: 4%), IPM (A: 5%; C: 8%), GEN (C: 3.1%), AMK (A: 3.1%), CIP (C: 3.1%). Essential agreement for MIC-based methods and scatterplots of MIC-agar versus zone diameter for the disk diffusion method were also determined.

Conclusion: Antimicrobial susceptibility testing systems were reliable in predicting *Aeromonas* susceptibility to AMX, CTX, FEP, GEN, TOB, AMK, CIP, OFX, NAL, but were unreliable in predicting susceptibility to AMC, TCC, TIC, CFT, IPM and such results should not be transmitted to clinician without control. Users should be aware of these limitations and improvement should be considered.

P812 Appropriate Muller-Hinton agar is crucial for the performance of metallo-β-lactamase E-test

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Objectives: Metallo-β-lactamases (MBLs)-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 (Becton Dickinson, bioMerieux, Difco, Oxoid) with the 9 phenotypic MBL-positive *Pseudomonas* isolates from the prevalence study.

Results: Of the 9 phenotypically MBL-positive strains (E-test with Difco 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 bioMerieux MH agar, with which two were missed (both VIM-2 producers). While the 6 MBL-negative isolates were detected correctly with bioMerieux MH agar, the number of false positive E-tests on other MH agars was the following: Becton Dickinson MH:1; Oxoid: 2; and Difco:6.

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

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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* J53 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 harboured armA. In cases of *S. marcescens* harbouring armA and its transconjugant (*E. coli* J53 harbouring armA), *S. marcescens* showed resistantance 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

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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 35º in CO2 was developed and wild-type distributions for major β-lactam drugs determined. Zone diameters were measured after 6 h.

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 0–20 h post signal for positivity were investigated.

Results: Reference strains and all clinical isolates grew sufficiently after 6 h. 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, eg. 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.

	Zone (mm) after 6 h incubation			
	n	ceftazidime	cefotaxime	cefoxitin
ATCC 25922	9	23–25	23–26	nd
E. coli non-ESBL	17	21–24	21–25	nd
E. coli ESBL	20	6–24	6–19	nd
ATCC 29213	9	nd	nd	19–20
MSSA	33	nd	nd	17–20
MRSA	20	nd	nd	6–17

P815 Evaluation of direct susceptibility profile testing by disk diffusion on respiratory tract samples

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Objectives: To compare direct susceptibility profile testing (DSPT), using a disk diffusion technology, on respiratory tract samples, with standard susceptibility testing (SST).
Methods: Respiratory tract samples, showing mainly staphylococci or Gram negative rods (GNR) on Gram stain, were directly swabbed onto Mueller-Hinton agar and paper disks for antibiotics active either against staphylococci or against Gram negative rods were applied. Standard culture was done on the samples and a standard disk diffusion test was performed on colonies grown overnight. A total of 208 samples, mainly from intensive care patients were analyzed.
Results: In 34/208 (16%) samples, culture showed no growth or growth of commensal flora. In 133/208 (64%) samples only one potentially pathogenic organism was recovered in culture, 5 of them concerned a pathogen unable to grow on Mueller Hinton agar. Of the remaining 128, 89 yielded GNR and 39 *Staphylococcus aureus*. DSPT was not interpretable in 12% of all possible micro-organism antibiotic combinations. Among the 1184 interpretable micro-organism-antibiotic combinations, there was a total agreement between DSPT and SST of 98.2%. The percentages of very major, major and minor errors were respectively 0.00%, 0.11% and 1.71% for GNR and 0.66%, 0.98% and 0.00% for staphylococci. In 41/208 (20%) samples more than one potentially pathogenic organism was recovered in culture. The combination of several GNR in culture occurred in 20 samples, the combination of one or more GNR and *S. aureus* in 21 samples. In the first combination 80% of all possible micro-organism combinations were interpretable; in the second combination only 42% (DSPT was only used for staphylococci or GNR). Results of DSPT were compared to the cumulative susceptibility of all pathogens. Among the 354 interpretable micro-organism-antibiotic combinations, the percentages of major and minor errors were respectively 1.46% and 4.39% for the combination of GNR and 2.01% and 2.01% for the combination of GNR and staphylococci. No very major errors were found. Even with incomplete or complex profiles, results of direct testing were useful in suggesting adequate antibiotic treatment.
Conclusion: Direct susceptibility profile testing on respiratory tract samples is potentially very useful in the management of critically ill patients, as results are obtained 24 hours earlier than conventional testing. However, additional conventional testing remains necessary.

P816 Determination of provisional British Society for Antimicrobial Chemotherapy zone breakpoints for 10-μg doripenem discs

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Objectives: Doripenem is a new carbapenem, recently licensed in the EU. A 10-IFN-ug doripenem disc has been proposed for routine susceptibility testing. We determined provisional zone breakpoints for these discs in the BSAC disc method.
Method: Isolates (n 800) were from the BSAC bacteraemia survey (2005–2006), supplemented with additional isolates with doripenem MICs outside the wild type distribution from other ARMRL survey collections and reference submission, care was taken to, whenever possible, select isolates with MICs straddling breakpoints. Zones and agar dilution MICs were determined by BSAC methods, and reviewed against European Committee on Antimicrobial Susceptibility Testing/European Medicines Agency (EUCAST/EMEA) MIC breakpoints of $S \leqslant 1$ mg/L and $R > 1$ mg/L for streptococci, and $S \leqslant 1$ mg/L and $R > 4$ mg/L for staphylococci, enterococci, Enterobacteriaceae, *Acinetobacter* spp. and *Pseudomonas* spp. Error minimisation was used to derive the breakpoints.
Results: Corresponding zone breakpoints for, IFN-a/IFN-b-haemolytic streptococci, and *S. pneumoniae* were $S \geqslant 23$ mm, $R \leqslant 22$ mm; for *S. aureus* and coagulase-negative staphylococci $S \geqslant 32$ mm, $R \leqslant 27$ mm, (though, non-susceptibility to doripenem here is best inferred from methicillin resistance); for *Enterococcus* spp., $S \geqslant 22$ mm, $R \leqslant 18$ mm; Enterobacteriaceae, $S \geqslant 24$ mm, $R \leqslant 18$ mm; *Acinetobacter*, $S \geqslant 24$ mm, $R \leqslant 17$ mm and *Pseudomonas* $S \geqslant 32$ mm, $R \leqslant 24$ mm.
Conclusion: The 10-IFN-ug doripenem discs effectively differentiated between S/I/R for most bacterial groups. For streptococci where there are currently few or no doripenem non-susceptible isolates reported, MIC determination (e.g. by Etest) should be performed on isolates producing zones close to the susceptible breakpoint.

P817 Calculation and validation of a discriminating disc content for colistin

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Objectives: Colistin (a polymyxin E) displays activity mainly against Gram negatives: Enterobacteriaceae (except for *Serratia, Proteus, Providencia*), *Pseudomonas* and *Acinetobacter*. This is a voluminous cyclic polypeptide with a lipidic chain of high molecular weight (1200 Da). This structure involves agar diffusion problems of the molecule and thus interferes with the diffusion methods. Several disk contents are recommended: 50 μg by the CA-SFM (Antibiogram Committee from the French Microbiology Society), 25 μg by the BSAC and 10 μg by the CLSI.
Methods: In this study, by using Vesterdal formula which connects the Log (MIC) with the square of the inhibition zone radius, a discriminating charge was calculated then validated by the diffusion method. In total, 250 strains were used: 130 clinical isolates (19 *A. baumannii*, 18 *C. freundii*, 20 *E. cloacae*, 21 *E. coli*, 19 *K. pneumoniae*, 33 *P. aeruginosa*), 120 mutant strains of reduced susceptibility obtained by the method of the antibiotic continuous gradient. MICs were determined by the agar dilution method. The parameter (1/4 DT) [D: diffusion coefficient; T: critical time] was calculated for all the strains by using 7 disc contents (2.5, 5, 10, 20, 30, 40, 50 μg)
Results: Values of 1/4 DT ranged from 0.019 to 0.114 with a median = 0.059, corresponding to a diffusion coefficient 3 times slower than those of β-lactams and fluoroquinolones. The contents equal or superior to 5 μg were not discriminating.
Conclusion: We recommend 2.5 μg content with S (susceptible; MIC ⩽ 2 mg/l), presence of an inhibition zone and R (resistant; MIC > 2 mg/l), absence of an inhibition zone.

P818 Impact of different EUCAST and CLSI interpretive breakpoints on antimicrobial susceptibility of *Pseudomonas aeruginosa* – SMART 2007

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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* of 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.

Drug	S/I/R* Breakpoint		% S/I/R*		P (%S)
	CLSI	EUCAST	CLSI	EUCAST	
Amikacin	16/32/64	8/16/32	87/4/9	74/12/14	0.0001
Cefepime	8/16/32	8/–/16	75/10/15	75/–/25	1
Ceftazidime	8/16/32	8/–/16	74/6/20	74/–/26	1
Ciprofloxacin	1/2/4	0.5/1/2	72/4/24	69/3/28	0.2
Imipenem	4/8/16	4/8/16	73/8/19	73/8/19	1
Levofloxacin	2/4/8	1/2/4	72/4/24	65/6/29	0.007
Piperacillin-tazobactam	64/–/128	16/–/32	85/–/15	76/–/24	0.0001

*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

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Objectives: To develop an automated method for estimation of a broad range of antibiotic susceptibilities given a restricted antibiogram.
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 antibiogram and cross-resistance, expressed as the conditional probability of resistance for non-tested antibiotics given the tested antibiotics. The method was derived using a bacteraemia database of 3347 clinically significant isolates (CSI) collected between 2002–2004 and validated on a bacteraemia 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 ($p \leqslant 0.10$). 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 ($p < 10^{-99}$).
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.

Susceptibility of *Acinetobacter* spp.

	% a priori coverage	Susceptibility	Odds for S $p \leqslant 0.10$	Odds for R $p \leqslant 0.10$
penbriti	3	R		
pipera	11	R		
keflin	2	R		
cefuroxi	4	R		
ceftazid	17	R		
ceftriax	3	R		
genta	25	R		
tobra	34	R		
amikacin	30	S	5.8	
chloram	17			
septrin	14	R		
oflo	15	R		
aztreona	3	R		
colistin	98	S		
tetra	10	R		
augmenti	5	R		
cipro	15	R		
minocycline	52	R		0.47
tazocin	14	R		
cefepime	18	R		
unasyn	87			
ertapenem	14			
imipenem	56			
meropenem	57	S	21.5	

P820 Assessment of Microscan® rapid ID-AST versus conventional panels for Gram-negative bacteria

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Objectives: The new rapid MicroScan® 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® 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® 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 carbapenemasis 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), meropenem (2/79), amikacin (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-esistant *Acinetobacter baumannii* in critically ill patients

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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 to reduce nosocomial infections with some of the Enterobacteriaceae, as intestinal colonisation is an important process in pathogenesis. In order to determine whether 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).
Methods: 48 stool samples and 23 perineal swabs obtained from ITU patients were plated on to Chromagar *Acinetobacter* (CAAB) and then inoculated into peptone broths. The agar plates were examined after 24 hrs for the growth of aqua blue colonies indicative of *A. baumannii*. The broths were incubated for 24 hrs and then used as a PCR template in an MDRAB specific (OXA-51, 23, 24 and 58) PCR. These results were compared with those obtained using the solid media. The clonal complex of all strains recovered on CAAB was determined using a multiplex sequence (csuE, blaoxa-51, ompA) based typing scheme.
Results: 34 samples were culture positive for MRAB 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 ($p < 0.05$). All isolates were found to be members of European Clone II.
Conclusion: 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

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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 a 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 18 h, and 100% at 24 h (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 (18–24 h) comparable to these 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

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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 vanB 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 $\geqslant$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.

Organism (no.)	Susceptibilities as determined by Vitek 2					
	Vancomycin			Teicoplanin		
	S	I	R	S	I	R
vanA VRE (12)	0	2	10	1	0	11
vanB VRE (13)	0	1	12	13	0	0
vanA+vanB VRE (1)	0	0	1	1	0	0
vanC1 VRE (22)	0	7	15	0	0	0
vanC2 VRE (11)	3	7	1	0	0	0
VSE (24)	24	0	0	24	0	0

S sensitive, I intermediate, R resistant.
VRE vancomycin resistant *Enterocccus*, 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

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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: 3.91×10^4, 95% CI: $\pm9.66\times10^2$), Short-En (mean CFUs/ml: 6.79×10^4, 95% CI: $\pm1.22\times10^3$), and ON-En (mean CFUs/ml: 2.31×10^5, 95% CI: $\pm1.16\times10^3$) (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.

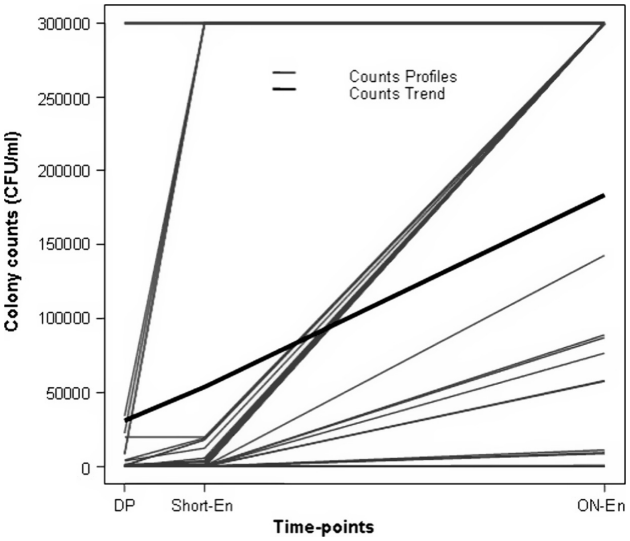

Figure: Colony count profiles and trend after DP, Short-En, and ON-En.

P825 Performance of Oxoid Brilliance MRSA, a new chromogenic medium

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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 35ºC. Growth of colonies showing blue coloration was 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

Isolate	No. of strains with a positive test result/total no. of strains (%)
MSSA	5/284 (1.8)
CoNS	9/265 (3.4)
Total (CoNS + MSSA)	14/549 (2.6)
MRSA	234/235 (99.6)

P826 Performance of three methods for determining the susceptibility to daptomycin and three other antimicrobials of *Enterococcus* spp.

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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.
Conclusions: Our findings indicate that both the E-test and Wider tests are reliable methods for routine microbiology laboratory practice to determine the antimicrobial susceptibility of enterococci isolated from clinical samples. These two techniques, however, often provide discrepant results when determining the MICs of daptomycin and vancomycin for *Enterococcus* spp. Nevertheless, this high rate of disagreement in no case translated to any type of interpretative error in the categorical comparison of test results with the reference results. The most likely explanation for this is the need to adjust the calcium cation concentration of the medium used when testing daptomycin, the impaired diffusion in agar media of both agents or use of an insufficient inoculum size. Thus, when faced with a serious enterococcal infection, a second MIC determination for daptomycin and vancomycin should be undertaken using a reference method such as the broth microdilution technique.

	Percentage strains with a MIC									
	⩾−2 dilutions		−1 dilutions		0		+1 dilutions		⩾2 dilutions	
	E-test	*Wider*	E-test	*Wider*	E-test	*Wider*	E-test	*Wider*	E-test	*Wider*
VAN	0	2	3.1	1	60.2	64.3	20.4	14.3	15.3	17.4
TEC	3.1	1	2	3.1	40.8	91.8	47.9	1	5.1	2
LZD	0	0	9.2	18.4	77.6	68.4	11.2	11.2	2	1
DAP	2	0	14.3	13.3	39.8	32.7	29.6	33.7	13.2	19.4

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. Burillo, 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 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.

	Percentage strains with a MIC									
	⩾−2 dilutions		−1 dilutions		0		+1 dilutions		⩾2 dilutions	
	MRSA	MRCNS	MRSA	MRCNS	MRSA	MRCNS	MRSA	MRCNS	MRSA	MRCNS
E-test										
VAN	0	0	0	10.2	40	71.2	53.3	18.6	6.7	0
TEC	0	1.7	3.3	22	20	40.7	70	30.5	6.7	5.1
LZD	8.4	15.3	31.7	32.2	53.3	50.8	5	0	1.7	1.7
DAP	0	3.4	28.3	16.9	46.7	54.2	23.3	23.7	1.7	1.7
Wider										
VAN	0	1.7	20	18.6	58.3	69.5	20	10.2	1.7	0
TEC	0	10.2	0	28.8	100	54.2	0	6.8	0	0
LZD	0	0	13.3	22	75	61	8.3	15.3	3.4	0
DAP	0	0	10	3.4	76.7	96.6	13.3	0	0	0

P828 An update to a comparison of E-test and agar dilution MICs for European anaerobes – a multi-year analysis

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Background: The Tigecycline Evaluation and Surveillance Trial (TEST) program is a global surveillance study designed to monitor activity of tigecycline (TIG) and comparators [clindamycin, penicillin (P), cefoxitin (CFX), metronidazole, pip/tazo (PT), meropenem (MER)]. This report compares Etest results from each site to agar dilution at a central lab.
Methods: 17 labs in Europe collected 2,040 anaerobic isolates between 2007–2008. MICs were determined locally by Etest, and at a central lab by agar dilution. %S by each method was compared using EUCAST breakpoints.
Results: The table lists the 3 drug/organism combinations tested that had >10% difference in % Susceptible (S) between agar dilution and Etest. All drugs not listed had %S by Etest and agar dilution within 10% of each other. Among individual species with n⩾10, however, there were 24/119 drug/organism combinations where %S by Etest differed by ⩾10% from the agar dilution result. In 21 of those cases Etest %S was lower than agar dilution.
Conclusions: Despite inherent difficulties often experienced with performing anaerobe susceptibility tests, in this study Etest results from 17 diverse labs across Europe yielded %S values broadly comparable to those obtained by a single reference lab using agar dilution. However, use of Etest in some specific drug/bug combinations such as *Bacteroides* spp. vs. CFX and PT, and *Clostridium* spp. vs. meropenem, may under-report susceptibility. Tigecycline Etest and agar dilution % S were comparable for all organisms tested.

Organism group	N	Drug	Agar Dil. %S	Etest %S
Anaerococcus spp.	45	P	68.2	90.2
Bacteroides spp.	430	CFX	85.5	57.8
Clostridium spp.	239	MER	100	89.5

P829 Are erythromycin and ciprofloxacin broth microdilution breakpoints for *Campylobacter* sp. suitable to categorise minimum inhibitory concentrations obtained by Epsilon test?

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Objectives: The Epsilon test (Etest) is a simple method to determine minimum inhibitory concentrations (MICs). The aim of the present

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 42ºC. Etest was performed according to manufacturer instructions; clinical isolates were incubated 24 hours at 42ºC; 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 37ºC and the other 24 hours at 42ºC.
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 $\leqslant$ 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 $\leqslant$ 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 37ºC 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 42ºC.
Conclusion: Our data indicate that CLSI interpretive criteria for BMD could be tentatively applicable to MICs obtained by Etest. Incubation at 37ºC 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 clarythromycin resistance in *Helicobacter pylori* strains isolated in St. Petersburg, Russia

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Objectives: Resistance to antibiotics quite often interferes with effective eradication of *Helicobacter pylori*. It is known that resistance to clarythromicin 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. Clarythromicin resistance of the strains was tested by the disk diffusion method. Region corresponding to 23S RNA was amplified by PCR, digested with MboII, BsaI and HhaI 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 clarythromicin and 4 out of 7 were resistant to digestion with MboII, BsaI and HhaI, 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; Posteraro R, 2006; Chihu L, 2005) but mutations C2195T and C2288T were found only together with A2142G or A2143G (Posteraro P, 2006; Pimbara E, 2007).
Conclusions: Molecular study of the structure of clarythromicin 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 clarythromicin 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

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Objectives: The treatment of patients infected with *H. pylori* is being seriously compromised due to increased antibiotic resistance patterns (e.g. amoxicillin, clarithromycin and metrodinazole). 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 Vitek 2 YST and AST-YS01 card for the identification and antifungal susceptibility testing of *Candida* spp.

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Objectives: The YST and AST-YS01 cards for Vitek 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 Vitek 2 (bioMérieux), using the YST and AST-YS01 cards, according to the manufacturer's instruction.
Results of identification and susceptibility testing with Vitek 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 falsly 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 card 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.

P833 Evaluation of the VITEK® 2 system for the susceptibility testing of *Candida* species and *Stephanoascus ciferii* with caspofungin, micafungin and posaconazole

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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 yeast.
Methods: Over 450 isolates were tested in VITEK 2 IUO 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*.

P834 Resistance to trimethoprim-sulfamethoxazole and *Tropheryma whipplei*

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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 folpP 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

P835 Plasmid analysis of ampicillin-resistant and ampicillin-susceptible *Enterococcus faecium* causing bacteraemia in a Spanish university hospital (1995–2008)

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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-SmaI 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-antitoxin 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%). ASEfm 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

pRUM (69%/5%), pCIZ2 (40%/20%) or pRE25 (21%/10%); and Axe-Txe (73%/5%). Relaxases from plasmids pHTβ and pRE25, as well as rep proteins from Inc18, pHTB and pAD1 plasmids were rare (all <5%).
Conclusions: AREfm were enriched in sequences associated with antibiotic resistances. Some sequences commonly present among VREfm CC17 were also found among most AREfm (from pRUM, pEF1 and pCIZ2 plasmids) while others (from Inc18 and pHTB plasmids) were scarcely observed in VSEfm. Endemic AREfm clones carrying a diversity of plasmids may supply a substrate for the acquisition of vancomycin resistance in our area.

P836 Dominance of ampicillin-resistant vancomicin-susceptible *Enterococcus faecium*-CC17 causing bacteraemia over a 14-year period in a university hospital, Spain

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Background: Bacteraemia 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/epidemicity 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 bacteraemia 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 bacteraemic 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 bacteraemia along 14 years associated with a dominance and persistence of related CC17 STs. These STs, enriched in IS16 element and esp and hyl virulence/epidemicity 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 country.

P837 High-density faecal colonisation by *Enterococcus faecium* in patients harbouring CC17

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Objective: To quantify the proportions of *E. faecium* (Efm) and *E. faecalis* (Efc) in faecal samples recovered from CC17-Ampicillin-Resistant Efm (AREfm)-colonised [AREfm(+)] and non-colonised [AREfm(–)] patients.
Methods: A faecal sample was recovered from 16 patients admitted in the Haematology and Gastroenterology/Nephrology ward at the Univ. Medical Center (Utrecht, The Netherlands) and this was used for bacterial quantification (July-Sept 2007). For each faecal sample ARE colonisation was confirmed using Enterococcosel Agar plates supplemented with ampicillin (16 mg/L). Presence of CC17 was determined by MLVA. The total number of Efc and Efm of each faecal sample were enumerated by Fluorescent In Situ Hybridisation (FISH) using specific probes (Waar et al. 2005) and compared with the total amount of cells (per gram wet weight) in the sample.
Results: 8/16 patients were AREfm(+) (mean age 64±10.1; 7 male) and 8/16 were AREfm(–) (mean age 49.6±19.4; 5 male) with a similar length of stay (22.5 and 20.5 days, respectively). Mean total number of cells was 8.6×10^9 and 1.5×10^{10} cell/g for AREfm(+) and (–) patients, respectively. Efm was undetectable among all AREfm(–) patients, whereas very high numbers of Efm were observed in all AREfm(+) patients (2.3×10^8, ranging 0.5%-7% of the total number of cells). 5/8 AREfm(+) patients presented a mean of 3×10^6 cells for Efc (ranging 0.01–0.52%) and in one AREfm(–) patient, Efc represented 5% of the total number of cells (1.3×10^8). In the rest of patients Efc fell under the detection limit (10^5) [3 AREfm(+) and 7 AREfm(–) patients]. All AREfm(+) patients received antibiotic treatment, most of them in combination [3rd gen. cephalosporins (4 patients); amoxicillin-clavulanate (3 patients); gentamicin (2 patients); carbapenems ciprofloxacin and metronidazol (1 patient)], whereas only one AREfm(–) patient received amoxicillin-clavulanate, gentamicin and ciprofloxacin.
Conclusions: This study demonstrated a higher Efm cell density than that of Efc in faeces from patients colonised with CC17-AREfm, while in non-CC17 colonised patients Efm are below the detection limit. High Efm densities could facilitate host-to-host transmission and hospital environmental contamination. Antibiotic-mediated increased Efm density and the ecological shift in the Efc:Efm normal ratio in the intestinal compartment might contribute to the current increasing proportion of Efm-CC17 in bacteraemic episodes.

P838 A nationwide outbreak of vancomycin-resistant enterococci in Sweden, 2007–2008

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Objectives and Background: The aim is to describe an ongoing nationwide outbreak of vancomycinresistant enterococci, vancomycin-resistant enterococci (VRE). The outbreak was first recognised in Stockholm county in October 2007 and has subsequently been reported from Västmanland, Uppsala and Halland counties.
Vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* (VRE) infection and carriage have been mandatory notifiable in Sweden according to the Communicable Diseases Act since 2000. Around 20–50 cases were reported annually between 2000–2006.
Methods: Analysis of all VRE-cases reported in the national web-based surveillance system SmiNet2. Genotyping and pulsed-field gel elecrophoresis (PFGE) performed at local laboratories and at the Swedish Institute for Infectious Disease Control, respectively.
Results: During the period July 1 2007 to December 11 2008 altogether 618 reports of VRE were received. 437 of the notifications came from Stockholm, 74 from Halland, 72 from Västmanland, 13 from Uppsala and 22 from another 8 counties throughout Sweden. The median age of the cases was 70 years. 486/618 of the cases were detected in faecal screening samples. Blood-stream infection was reported in 12 cases.
E. faecium with gene vanB (EfmB) was found in 465 cases and *E. faecium* with vanA in 90 cases. The remaining isolates have not yet been fully typed. PFGE has shown that virtually all EfmB isolates analysed until now belong to the same clone.
Conclusion: A nationwide outbreak of *E. faecium* with vanB traced back to August 2007 is ongoing in Sweden. The reasons for the extensive

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.

P839 Isolation of glycopeptides-resistant enterococci from stool and blood samples of hospitalised patients during a three-year period

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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 bacteraemia. 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 631/5047 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 bacteraemia cases was: Haematology ward 5, Gastroenterology ward 3, ICU 12, Internal Medicine department 6, PBU 9, Vascular Surgery department 1. Six cases of GRE bacteraemia 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 carriage led to bacteraemia. Antibiotic policy and strict enteric precautions should be implemented to restrict GRE carriage. As the risk of GRE bacteraemia exists the epidemiological surveillance is necessary especially for immunocompromised patients.

P840 International spread of vancomycin-resistant *Enterococcus faecium* (CC17, CC5) and *Enterococcus faecalis* CC2 among animals and humans

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Objectives: Spread of vancomycin resistance among enterococci (VRE) by clonal expansion or by horizontal transfer has been described in both hospital and community settings. However, transmission between animals and humans has been scarcely demonstrated, probably due to the apparent host specificity observed for enterococcal clonal complexes and for some genetic elements. We analysed a collection of wide disseminated VRE from humans and swine in order to assess possible routes of genetic exchange and transmission.
Methods: We characterised 6 *E. faecium* (VREfm) and 1 *E. faecalis* (VREfc) from swine, the only expressing resistance to glycopeptides among 473 collected from piggeries in different regions of Portugal (2006–07). Epidemic clinical isolates from European hospitals were included for comparison. Susceptibility profile, resistance and virulence genes (PCR), clonality (PFGE, MLST), transferability (filter mating) and Tn1546 (PCR overlapping, I-CeuI-hybridisation) were characterised. Plasmid analysis included determination of size and content (S1-nuclease) and comparison of ClaI-RFLP patterns.
Results: We identified two VREfm and one VREfc PFGE types persistently recovered from humans and also observed in swine. One VREfm (ST132-CC17) was collected from swine (n = 1) and hospitalised patients (n = 2) in Portugal (PT), all sharing similar antibiotic resistance and virulence profiles, and a 70 kb plasmid with the same RFLP profile. The other VREfm (CC5) was a clone widespread among swine from 4 EU nations (Portugal, Spain, Italy, Denmark) since 1997 and also identified from 3 patients of different PT hospitals (2002). All CC5 isolates (ST5, ST6, ST185) contained the variant D of Tn1546 type located in a 150 kb plasmid. The VREfc (CC2, ST6) is the epidemic multidrug-resistant clone "B", widely disseminated in PT, Spanish and Italian hospitals since 1993 and now recovered from swine. VanA plasmids from clinical isolates showed variable sizes and RFLP patterns, though an identical 100 kb plasmid was observed in the PT swine isolate and in one clinical isolate from Spain (Tn1546 type A; same RFLP).
Conclusions: We described VREfm and VREfc identical strains within major enterococcal CCs from swine and humans, stressing out the role of animals as reservoir of VRE involved in human infections. This is the first report documenting the presence of CC2 Efc strains outside the hospital setting and identical VanA plasmids in swine and humans from different countries.

P841 Plasmid analysis of vancomycin-resistant *Enterococcus faecium* isolates from hospitals and aquatic environments in Portugal (1996–2008)

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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 analyzed 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/ClaI-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 ISEf1 or IS1216, 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%), pCIZ2 (80%) or pHTB (33%); rep proteins from pRUM (88%), pCIZ2 (65%), pRE25 (64%), Inc18 and pHTB (36% each), pEFNP1 (33%); Axe-Txe (16%) or omega-epsilon-zeta systems (14%). Relaxases from pAD1 (8%) and pRUM (4%), as well as rep proteins from pAD1 (7%) and pDT1 (4%), were rarely detected. VanA-plasmids were predominantly derivatives of pEF1 plasmid containing rel-pEF1, mostly associated with rep-pRUM and eventually with rep from pRE25 and/or Inc18. 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

our area. The conjugative plasmids driving the spread of vancomycin resistance in our area are mosaics including mainly sequences from pEF1 and pRUM.

P842 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

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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 pos-intervention period. Chi-square test was used to compare data pre and pos 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 pos-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.

P843 Vancomycin-resistant enterococci infection outbreak in the intensive care unit

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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 (broth 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 $\geqslant 32\,\mu g/ml$ for both glycopeptides. E-test revealed vancomycin's MIC $> 256\,\mu g/ml$ and teicoplanin's MIC=$32\,\mu g/ml$ in all strains. All strains were susceptible to linezolid. Molecular method (PCR) detected VanA gene in both two 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.

P844 Typing of vancomycin-resistant enterococci from Danish hospitals

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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 vanB gene ($n = 15$) and the remaining isolate contained the vanA 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.

P845 Vancomycin-resistant enterococci in haematopoietic stem cell transplant recipients

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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 venious 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

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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 the same 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% of strains), 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 addresss the clinical choice according to the local epidemiology and antimicrobial testing features.

P847 Prevalence of vancomycin-resistant enterococci in presistance patients who referred to a paediatric hospital, Tehran, Iran

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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 Mofeed 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 Mofeed 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 Enterococcocel agar and Bile Esculin agar. And then some specific tests were performed for their confirmation such as PYR test, growth in 15ºC and 45ºC in Mueller Hinton agar. Growth in 6.5% NaCl. E-test was performed on VRE strains. Selected antibiotics for E-test were included cephotetan, rifampicin, gentamycin, ciprofloxacin, ceftriaxon, oxacilin, 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.

Nosocomial outbreaks and potential sources of infection

P848 Nosocomial outbreak of an extended-spectrum β-lactamase producing *Klebsiella pneumoniae* strain on a medical intensive care unit

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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 $\geqslant$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, supposably 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

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Objective: 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.
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.
Conclusions: To our knowledge this is the first reported outbreak that provides some evidence that food can be a transmission vector for ESBL-producing-KP in the nosocomial setting.

P850 Environmental contamination in the rooms of ESBL-colonised patients

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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 48 h at 37ºC. Species identification of Gram negative bacteria was performed using the API 20 E® (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 Xba1.
Results: ESBL positive enterobacteria were present in the rooms of only 8 of 36 patients colonised with ESBL positive enterobacteria (22%). Contamination of HCWs' 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

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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 in 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

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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 uricult. ESBL was mainly diagnosed in clinical samples, six patients were screened for ESBL

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); cefpodoxime, ceftriaxone, ceftazidime 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 80% (52/65) 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 (73% 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.

P853 The extended antibiogram, a useful tool for typing of ESBL producing *E. coli*

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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 between isolates following overnight incubation.

Method: A collection of 51 ESBL producing *E. coli* isolates, including 6 and 9 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 comparision 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

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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 urinary 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=1.86–25.21, 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.

P855 Report of an outbreak of carbon dioxide dependent methicillin-resistant *Staphylococcus aureus* on a hospital ward

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Objective: To investigate and control an outbreak of carbon dioxide (CO2) dependent meticillin 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 (Slidex 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 37oC in 5% CO2, however, a heavy growth of an isolate with colonial morphology typical of S.aur 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 meticillin resistant S.aur (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-SCCmecIV).
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.

P856 An outbreak of multidrug-resistant *Pseudomonas aeruginosa* sepsis after endoscopic retrograde cholangiopancreaticography

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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 cholangiopancreaticography (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 from service and underwent gas sterilisation with ethylene oxide.
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.

P857 Investigation of nosocomial outbreak of multiresistant *Acinetobacter* spp. at a Korean hospital

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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 SmaI-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.
Additional samples with positive cultures were: 3 of 24 humidity jar of individual patients, and 3 of 44 hands of ICU nurses. Isolates with multiresistance to all 13 antimicrobial agents tested were randomly selected and used to compare PFGE patterns of SmaI-restricted genomic DNA. An identical pattern (A1) was observed in 12 (42.9%) of 28 patient isolates, and 11 (39.3%) of 28 environment isolates. Intensive cleaning of respiratory equipments and fomites in the ICUs with antiseptic solutions, and enforcing hand washing resulted in a slight decrease of *Acinetobacter* isolation to 137 in November.
Conclusion: 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.

P858 Outbreak of *Burkholderia cepacia* in two German university hospitals caused by contaminated prefabricated washcloths

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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 mouthwashs, 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, SpeI 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 pacer 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.

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

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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 identical antibiotic resistance profile, all were sensitive to β-lactames. PFGE profiles of the clinical isolates showed high similarity to the environmental strains.

In conclusion, typing results supported the hypothesis that tap water and/or taps are likely reservoirs of infective strains. Point-of-use filters were found to be effective means for the infection control of pseudomonads.

P860 Prevalence and antibiotic resistance of *Stenotrophomonas maltophilia* in the water supply of a haematology ward

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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 filtrates 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 BSAC breakpoints for *Pseudomonas*, 5.6% were resistant to colistin, 25.6% to ceftazidime, 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 $\geqslant 3$. Nine isolates were resistant to 6 drugs.

Conclusions: Hospital 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

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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 ($p = 0.73$).

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

Hosp	First sample	Measures taken					Second sample	p
	(pos/total)	a	b	c	d	e	(pos/total)	
1	2/9	yes				yes	2/9	1.0
2	1/3					yes	1/1	0.4
3	3/3		yes				1/1	n/a
4	12/12	yes		yes	yes		6/6	n/a
5	0/4						0/6	n/a
6	4/7						0/10	0.04
7	1/4						0/6	0.33
8	2/2						0/2	0.14
9	2/10	yes	yes	yes			2/4	0.28
10	1/12				yes		0/17	0.37
11	1/1						1/1	1.0
12	5/6		yes				15/21	0.56
13	1/13		yes	yes			0/13	0.48
14	5/11			yes			4/9	0.96
15	5/12		yes				3/12	0.39
16	3/18	yes	yes	yes	yes		n/a	n/a
17	18/32				yes		20/32	0.61
18	8/34				yes		9/36	0.89
19	2/5		yes	yes	yes		1/11	0.17
20	2/6		yes	yes	yes	yes	0/6	0.24
21	1/9						6/9	0.03
22	1/5					yes	1/4	0.86
23	0/7						1/8	0.52
24	4/4	yes	yes	yes	yes		6/6	n/a
25	2/2	yes	yes				6/6	n/a
26	4/9			yes			2/9	0.32
27	2/16						1/16	0.55
28	2/4						4/4	0.21
Total	94/260						92/265	0.73

Measures: (a) Technical check of the water system; (b) periodical heating of complete system; (c) constant increase of temperature above 60ºC; (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.

P862 Distribution of environmental *L. pneumophila* sg.1 strains with heterogeneous chlorine-susceptibility patterns inside hospital plumbing system

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Objectives: To describe the distribution of *Legionella* strains and their evolution during the application of a five-year Water Safety Plan (WSP) in a university hospital based on a disinfection-filtration strategy.
Methods: Between March 2002 and June 2008, 224 out of 698 water samples were positive for *Legionella* spp.; 91 strains were selected on temporal and spatial criteria and were analysed applying both the comparison of the nucleotide sequences of six genes by SBT or the comparison of the genomic profile by PFGE. The chlorine-susceptibility of representative isolates was assessed according to the BS EN 1040:1997. Feasibility of a rapid assessment of chlorine-susceptibility using quantitative ATP detection was also evaluated.
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.

P863 Use of soda fountains in hospitals: four-year surveillance of daily routine

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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 22ºC; >20 CFU/ml at 36ºC), 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.

P864 Evaluation of bacterial contamination of patients using ocular drops

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Objectives: The aim of the present study was to investigate the incidence of bacterial contaminations of in-use eye drop products in the teaching department of ophthalmology, Imam Khomeini Hospital, Ahvaz, Iran.
Methods: Two hundred and eighty seven eye drop bottles were randomly collected at the end of day 1, day 2, day 3, day 4 and day 7 of use. The eye drop residues, swabs from internal caps and droppers were inoculated onto MacConkey agar and blood agar. The identification of the recovered organisms was accomplished using standard microbial identification techniques.
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. The most contaminated part of the eye drop products was the caps (45.9%) followed by droppers (41.0%) and residual contents (13.1%). Considering mendicants contents, those with pilocarpine (41.7%) had the highest rate of contamination followed by atropine (31.8%), tropicamide (28.6%) and betamethasone (23.3%).
Conclusion: Our study revealed the potential risk of contamination of in-use eye drop products in hospitals, but we did not find a direct relationship between usage duration and contamination rate.

P865 Horizontal transmission of group B streptococci in a neonatal intensive care unit

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Group B *Streptococcus* (GBS) is a leading cause of life-threatening infections (pneumonia, septicaemia, meningitis) in newborn infants and it can result in death or long-term disabilities. Early onset disease occurs within the first week of life, with most cases appearing within 72 hours and it is normally related to vaginal carriage in the mother and subsequent vertical transmission during birth. The cause of late onset disease, occurring after the first week of life, remains unclear: pathogens may have originated from the maternal genital tract (vertically transmitted), but also may come from contacts with human carriers or nosocomial sources after birth (horizontally transmitted).
During a 20 days period (4–23 May 2008), two preterm newborn infants (case nº2 and case nº3) developed late-onset neonatal GBS infection while hospitalised by the Neonatal Intensive Care Unit (NICU) of the Maternity Hospital "G.Salesi", Ancona, Italy. The onset of these two cases was preceded by a case of early-onset GBS neonatal disease occurred in a baby hospitalised in the same ward with a culture proven GBS sepsis (case nº1 – supposed index case).
Objective: to assess the possible cross contamination within the NICU
Methods: to define the clonal relatedness of the three GBS strains isolated from the blood of the three babies with GBS sepsis, we used pulsed-field gel electrophoresis (PFGE): DNA was extracted and digested with 40 U of SmaI, the PFGE profiles of the isolates were compared according to Tenover et al.'s criteria.
Results: 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).
Conclusions: Even if it is not frequently reported, horizontal transmission of GBS can represent an actual risk for babies during hospitalisation. These findings suggest us to perform further studies for identification and assessment of unexpected GBS infection cases.

P866 Faecal carriage of carbapenem resistance in Hong Kong

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Objectives: To document the prevalence of carbapenem-resistant organisms in stool samples in Hong Kong.
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 35ºC 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.
A total of 53 (2%) strains that were imipenem-resistant and belonging to six species were obtained with 37 (70% of the 53) being Stenotrophomnas maltophilia. The others were *Klebsiella pneumoniae* (1), *Acinetobacter baumannii* (9), *Pseudomonas aeruginosa* (3), Cedecea 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 Cedecea lapagei strains were from patients who were recently admitted into the hospital.
Conclusions: Carriage of carbapenem-resistant organisms was uncommon in Hong Kong. Continuous surveillance to monitor the development of carbapenem-resistance especially in naturally carbapenem-susceptible organisms should be carried out to prevent spread of the resistance.

Diagnostic methods

P867 Evaluation of a sonication protocol for the detection of bacteria in retrieved osteosynthesis implants

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Objectives: To evaluate the usefulness of a sonication protocol to detect the presence of bacteria in retrieved osteosynthesis implants from patients with and without clinical infection.
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. intermedius*, 1 *S. maltophilia*, 1 *P. stuartii*, 1 *P. prevotii*, 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. acnes* (from the same patient), 1 *R. pickettii*, 1 *S. paucimobilis*, 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 significatively 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

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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. Trampoz 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 ± 1664.10 and 32357.29 ± 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 staphylococci 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: CFU counts and bacterial identifications of isolates

Case no.	Before sonication	After sonication
1	3000 CFUs *Pseudomonas aeruginosa*	50,000 CFUs *Pseudomonas aeruginosa*
2	No growth	5000 CFUs MSSA*
3	No growth	8000 CFUs MSSA
4	40,000 CFUs MRSA	100,000 CFUs MRSA**
5	No growth	No growth
6	1 CFU MRCNS	50,000 CFUs MRCNS***
7	30,000 CFUs *Pseudomonas aeruginosa*	80,000 CFUs *Pseudomonas aeruginosa*
8	No growth	30,000 CFUs non-fermentative bacilli
9	40,000 CFUs MRSA	70,000 CFUs MRSA
10	No growth	No growth
11	No growth	No growth
12	No growth	No growth
13	No growth	6000 CFUs *Pseudomonas aeruginosa* and *Enterobacter* spp.
14	No growth	2 CFUs MRCNS

*Methicillin-sensitive *Staphylococcus aureus*.
**Methicillin-resistant *Staphylococcus aureus*.
***Methicillin-resistant coagulase-negative staphylococci.

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

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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 Medicin 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 leucocytes in pus or tissue plus local signs and symptoms and/or systemic markers of inflammation (fever, leucocytosis, increased C-reactive protein)

Results: We investigated 60 patients with 41 septically 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 polyethylen-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 osteosynthesis 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 endoprosthesis 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

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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 punction with a Mallarmé needle was performed. Bone aspiration was inoculated in Bactec® haemoculture aerobic and anaerobic bottles.

Three out of 36 punctions were excluded because antibiotics were started before the punction. Fourteen punctions (42%) were negative with a good 3 months outcome without antibiotics. Nineteen punctions (58%) were positive: one *Streptococcus*, twelve coagulase negative *Staphylococcus* and six *Staphylococcus aureus* (three MRSA). All cultures were monomicrobic 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 spoting when available.
Conclusion: The coupled procedure, gallium SPECT-CT scan-bone aspiration with Mallarmé needle is efficient to diagnose bone osteitis in diabetic feet. Gallium SPECT-CT scan provides a functional and exact imaging of bone inflammation when punction 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 quantative survival of *Escherichia coli*, *Streptococcus agalactiae* and *Candida albicans*

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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 nylon-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* (ATTC 25922), *S. agalactiae* (ATCC 13813) and *C. albicans* (ATCC 90028) after 0, 6, 24 and 48 hours preservation at 4ºC. 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 count 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 4ºC. 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 4ºC 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 4ºC). *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 4ºC 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

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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 100uL 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 24 h, refrigerated for the following 72/96 h and plated every 24 h.
Results: Colonies count was recorded for each strain for all storage times. HI in ES was viable up to 120 h but negative in TS. SP, BF, SPY, PA and PAC were stable at 120 h in ES; one log reduction was found for SP at 48 h, for BF, SPY and PA at 96 h in TS. CA, MRSA and VRE were stable up to 120 h 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 96/120 h 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

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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 microliters 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 from 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

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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 of the 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 10microliter 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/microliter) 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

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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 37ºC in air. Culture results were reported as no growth; urine contaminated if there were 3 or more kinds of colonies without a dominant species; and were considered positive if they contained $\geqslant 10^5$ or 10^4 to $<10^5$ colony-forming units/mL (cfu/mL) of an urinary pathogen of pure culture, if two or more potentially pathological bacterial species were isolated when the individual counts were $\geqslant 10^4$ cfu, or when the count for one organism was $\geqslant 10^4$ 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/ul and 25 bacteria/ul count as the cutt-off compared with bacterial culture.

Table 1.

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
Urine samples selected for culture (%)	488 (62%)

Conclusions:
1. These cutt-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

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Objective: Evaluation of diagnosis 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 November 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: 252–4). 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" (Nigrovic 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 (Se:97/80 Sp:32/75), CSF leucocytes >10/mm^3 with >50% of PMN (Se: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 theses 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.

P877 BacT/Alert automated blood culture system for culturing sterile body fluids and deep site abscesses

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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 16 (12%) 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) respectevely, 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.

P878 Liquid-based microbiology and automation: a new frontier in the management of bacteriology laboratory

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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 Amies 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.

P879 Identification of clinical isolates of *Bacteroides* by matrix-assisted laser desorption/ionisation mass spectrometry

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Objectives: Members of *Bacteroides* genus, are important anaerobic pathogens causing sever 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) $\geqslant$ 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*.

P880 Identification of members of the *Bacteroides fragilis* group by mass-spectrometric discrimination

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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. merdae* 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.

P881 Matrix-assisted laser desorption ionisation time of flight mass spectrometry is superior to biochemical identification in clinically important *Staphylococcus* species

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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 proteom 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 challanging, because some strains morphologically resemble *S. aureus*, and further tests are required (e.a. ornithin decarboxylase [ODC], pyrrolidonyl arylamidase [PYR]).
Methods: The species differentiation of 215 clinical isolates of *S. aureus* (70 methicillin suseptible and 105 methicillin resistant, 39 methicillin suseptible 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: Bacterial differentiation of staphylococci using MALDI-TOF MS technology is superior to biochemical tests. Further tests have to show analytical equivalence of this database to molecular biological methods. MALDI-TOF MS technology could be an excellent compensation for the time consuming and expensive gold-standard (sodA-sequencing, rpoB-sequencing or 16S sequencing).

P882 Identification of bacteria and yeast by matrix-assisted laser ionisation/desorption time-of-flight mass spectrometry (MALDI-TOF MS) in routine laboratory setting

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Objectives: 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 Sabouraoud'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.

P883 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

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Objectives: Enterococci are part of the human physiological intestinal flora, but gained an increasing relevance as a pathogen. Enteroccus 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 a 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

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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 Enterobaceriaceae 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 (AnagnosTec, 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

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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

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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 BioTyperTM 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 aerobical 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 BioTyperTM 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 BioTyperTM 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, 6.3%): 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.

P887 New automated solution for plate streaking: comparative evaluation of the PREVI Isola in a microbiology lab

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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 Prepoured 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, regarding the number of isolated, further processable colonies, the PREVI Isola was superior to the manual method.

For the 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.

P888 Usefulness and reproducibility of sputum Gram stain in LRTIs

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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 coccobacilli 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 coccobacilli identification was very high. Sputum Gram slide interpretation was reproducible and predictive of a positive culture.

P889 Performance of the PREVI™ colour Gram automated staining system

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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 decolorisation 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 carrousel. 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 decolorisation 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 Color GRAM using the Decolorizer 3 provided 100% and 98% agreement with the manual method for reference strains and clinical samples respectively. Discrepancies analysis was never in discredit of the automatic technique.
Conclusion: Even using a manual standardised protocol, significant staining differences may persist between operators. The PREVI Color GRAM system provides distinct and reproducible results, thus contributing to stain standardisation, besides saving dyes and technician time.

P890 The PREVI Isola system for automated streaking of patient materials

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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.

P891 Correlation of Sedimax® (Menarini) versus reverse light microscopy for the examination of urine sediment

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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 erythocytes 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 urine.
Concordance for leuco- and erythrocytes between both methods was respectively 73.1 and 82.8%; 26.9% of the urine samples showed major disconcordances 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 clumbed 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 disconcordance of white and red blood cells, the Sedimax® can not be used in our laboratory without further adjustments.

P892 In vitro effects of iodinated contrast agents on bacterial growth

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Objectives: Injection of iodinated arthrographic contrast medium in (artificial) joints (puncture arthrography) is used to diagnose infection or aseptic loosening of arthroplasties. 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 aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*. Inhibition was determined using a disk diffusion technique and a time-killing curve method in which high (5×10^8 CFU/ml) and low (5×10^3 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 35ºC.
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.

P893 Fever of unknown origin: differential diagnosis between infectious and non-infectious causes

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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. Data were prospectively gathered in two distinct, observational phases: an internal developmental study (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 56.5±11.2 years, 55% males, fever duration before admission 32.1±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.

P894 The diagnostic accuracy of endotracheal aspiration and bronchoalveolar lavage in the diagnosis of ventilator-associated pneumonia

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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 24 h.
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 72 h, 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.

P895 Utility of alveolar and ascitic sTREM-1 to diagnose aetiology of acute respiratory distress syndrome

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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 asses 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 ($p < 0.001$) 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 (sensibility 63%, specificity 100%) whereas a ratio <2 strongly indicated an abdominal infection (sensibility 89%, 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.

P896 Procalcitonine in abdominal surgery: is there an economical interest?

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Objectives: Sepsis 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 co-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 criterias of systemic inflammatory response (Temperature >38ºC or <36ºC, WBC count >12,000/mm^3 or <4000/mm^3, 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 (Gc: 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, Wilcox 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 ($p = 1$). PCT increased significantly in all of the 15 patients ($p < 0.05$). For 6 patients in Gc, a bacterial identification allowed to guide a cheaper antibiotic choice compared to the Gs ($p < 0.01$) where empirical antibiotherapy appeared to be more expensive.
Conclusion: It is not the systematical prolonged antibiotherapy 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 antibiotherapy. PCT could help to choose which patient would benefit from prolonged post-surgery antibiotics.

P897 Plasma procalcitonin levels in clinically stable patients with cystic fibrosis

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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.

P898 Changes in platelet sizes as indicators for clinical outcome of bacteraemia

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Objectives: Systemic infections such as bacteraemia induce acute phase responses, leading to multiple alterations including the haematostatic 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 bacteraemia.
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 mean platelet volumes (MPV) were tested during the three periods; the first period, between 30 days and 7 days before the onset of bacteraemia, 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 bacteraemia serve as prognostic indicators for bacteraemia.

P899 Lymphocytopenia and neutrophil/lymphocyte count ratio predict bacteraemia better than conventional parameters in an emergency care unit

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Objectives: Prediction of bacteraemia is essential in clinical decision-making. Absolute lymphocytopenia (lymphocyte count $<1.0\times10^9$/L) has been reported as predictor of bacteraemia 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 bacteraemia 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 bacteraemia than routine parameters like CRP, WBC or neutrophil count.

Table 1. Predicting bacteraemia using conventional parameters (CRP, WBC and neutrophil count), lymphocytopenia and the neutrophil/lymphocyte count ratio

Parameter, mean±SD	Cases, n = 92	Controls, n = 92	ROC area
CRP	176±138 mg/L	116±103 mg/L	0.62
WBC count	$13.6\pm6.6\times10^9$/L	$12.9\pm5.2\times10^9$/L	0.53
Neutrophil count	$12.1\pm6.1\times10^9$/L	$10.7\pm5.1\times10^9$/L	0.58
Lymphocyte count	$0.8\pm0.5\times10^9$/L	$1.2\pm0.7\times10^9$/L	0.73
NLCR	20.9±13.3	13.2±14.1	0.74

Fungal diagnostics and pathogenesis

P900 Development of PCR Electrospray ionisation mass spectrometry for identification of clinically relevant fungi and yeast

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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, *Coccidioides immitis/posadasii*, *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 Saccharomycete 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.

P901 Detection of candidaemia in patients with and without underlying haematological disease

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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 arabinitol, 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 arabinitol ratio in serum and urine was determined by gas chromatography (Larsson L, 1994). Antigen (Ag) was detected by the CandTec Kit (Ramco Laboratories Inc., Vernn, Houston, USA) and by using the Platelia *Candida* Ag ELISA kit (Bio-Rad, France) and anti-Mannan antibody (Ab) was detected using Platelia *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 arabinitol ratio in serum: 41.7%, 86.4%; CandTec *Candida* Ag: 66.6%, 70.8%. By lowering the cut off values to Mannan Ag: 0.10 ng/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 arabinitol ratio to only patients with haematological neutropenia 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 arabinitol ratio seemed useful in the setting of haematological underlying disease and neutropenia.

P902 *Candida* mannan and anti-mannan in the diagnosis of invasive candidal infections in neutropenic patients

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Objective: Antifungal (AF) therapy given to all pts with febrile neutropenia (FN) is costly and toxic. Beta-D-glucan assay is fungal-non-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 the 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), hepatosplenic candidiasis (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 $\geqslant$ 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 ⩾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.

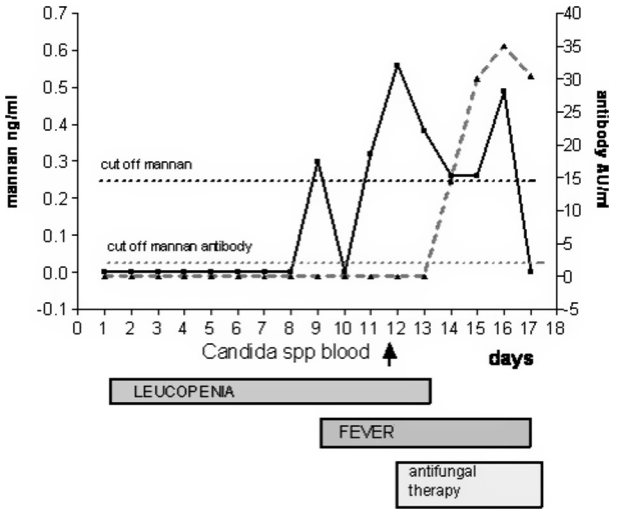

Figure: Serial course antigen and antibody pt#12.

P903 Adherence of *Candida albicans* on FEP (polymer of tetrafluoroethylene and hexafloropropylene) catheter and polyurethane catheter in an in vitro dynamic adhesion model

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Objectives: To investigate adherence features of *Candida albicans* in a dynamic adhesion model and to evaluate the impact of different catheter material on *Candida albicans* adhesion.
Methods: We designed an in vitro dynamic adhesion model to test the adherence features of Candid albicans to catheters of different material (Figure). Yeast-peptone-dextrose (YPD) broth was used as the flow medium. The flow rate was kept at 90 ml/min to mimic the average flow rate of human venous blood. FEP and polyurethane catheters were placed inside the glass tube. *Candida albicans* SC5314 at an initial concentration of 10^5 cell/ml was used. The whole model was maintained at 37ºC. When *Candida albicans* SC5314 growth reached mid-log phase (6 h), FEP and polyurethane catheters (both 2 cm long, outer diameter = 1.3 mm) were picked up and gently washed thrice with 1 ml phosphate buffered saline (PBS). This is followed by sonication in 1 ml PBS solutions at a frequency of 37kHz at 4ºC. The sonication procedure consisted of a 3 min sonication followed by 10 s vortexing, this was repeated thrice. After sonication, PBS solutions were used for viable count to calculate adherent cell number on the surface of catheters. Catheters were cultured in YPD broth to check whether sonication process was complete or not. Adherence features of different catheter materials were presented as the number of colony forming units (CFU). The experiment was done in duplicates. Two independent experiments were performed.
Results: At mid-log phase (6 h, 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.

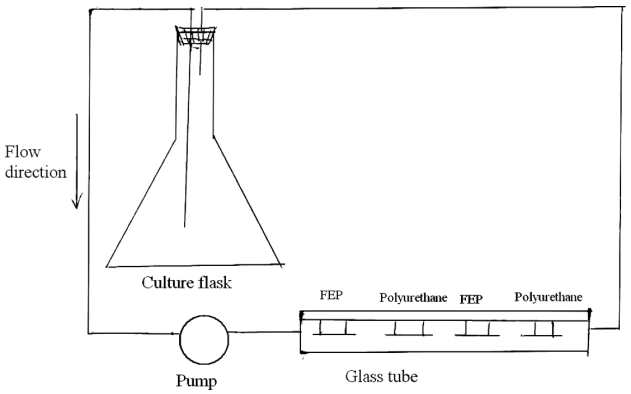

Figure: An in vitro dynamic adhesion model.

P904 Evaluation of commercial phenotypic identification systems for the characterisation of common and rare yeast pathogens

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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 (18 h versus 48–96 h 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.

P905 Anti-aspergillus antibodies and galactomannan antigen detection for serodiagnosis of aspergillosis

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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 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* 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, IgM and IgA, 3 (8.6%) for GM, IgG and IgA, 3 (8.6%) for GM, IgG and IgM, 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.

P906 Galactomannan as a prognostic marker for poor outcome of invasive pulmonary mould infection in patients with haematological malignancies

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Objectives: Little is known on prognostic factors for outcome of invasive mould infections in patients with haematological malignancies. Besides new prophylactic regimes, knowledge of predictors for therapy failure might improve outcome.
Methods: We analyzed cases of proven and probable invasive pulmonary mould infection in patients with haematological malignancies treated in a tertiary-care hospital from 2002–2008 with the aim to investigate risk factors for poor outcome.
Results: In 34 patients with probable (n = 18) and proven (n = 16) invasive pulmonary mould infection a galactomannan value of $\leqslant$ 0.5 ng/mL at the time of diagnosis was an independent risk factor of poor outcome at 6 months after diagnosis (HR 1.24, 95% CI 1.09–1.52, $p \leqslant 0.023$). Age, gender, underlying disease, transplantation, use of steroids, duration of neutropenia, CT findings, surgery and antifungal treatment had no significant influence on the outcome. Under an antimould treatment (and thoracic surgery in 16 patients) a radiological resolution was seen in 20 patients (59%) after a median of 80 days. Cure at 6 months was reached in 24 patients (70.6%). Overall 23 patients (67.6%) died during the follow up. Three patients died of a mould infection corresponding to an attributable mortality of 8.8%.
Conclusion: The level of galactomannan at diagnosis of the fungal infection is predictive for poor outcome.

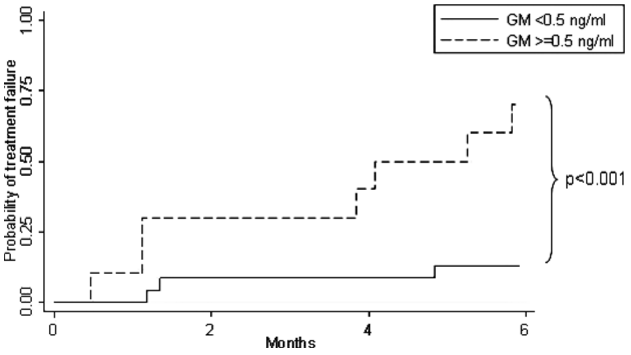

Figure 1. Kaplan-Meier curve of treatment failure within 6 months after diagnosis of invasive mould infection according to the initial galactomannan (GM) value.

P907 Evaluation of serum *Aspergillus* galactomannan in haematological patients

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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 reactives 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

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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

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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 jirovecii* colonisation

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Background: The accepted current diagnostic standard for *P. jirovecii* infection is the demonstration of the microorganism in sputum or bronchoalveolar lavage (BAL) samples using conventional stain or polymerase chain reaction (PCR). Besides oropharingeal washes (OWs) are used to detect *P. jirovecii* colonisation by nested PCR in general population. Several studies have evaluated the use of Real time PCR to detect the MSG genes of *P. jirovecii*.
The efficacy of this assay at mt LSU rRNA gene of Pneumocystis to detect the colonisation status is was not being proved.
Objective: To evaluate the usefulness of Real time PCR versus nested PCR using to detect *P. jirovecii* colonisation.
Methods: A total of 100 OWs specimens which included 23 samples colonised where *P. jirovecii* 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. jirovecii* 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 94.11% for the detection of *P. jirovecii* 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 analised.

P911 Hyper-pigmentation of blastospores in contrast to basidiospores of pathogenic cryptococci upon induction to ultraviolet radiation of near solar spectra

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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 neoformans* 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 suspended 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 neoformans* variety gatti blastospores was observed to be hyper pigmented compared to *Cryptococcus neoformans* variety grubii and *Cryptococcus neoformans* variety neoformans.
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 neoformans* variety gatti blastospores points to the increased virulence and and its ability to infect an apparently immunocompetent host.

P912 Fungal metabolites can fuel an underlying HIV infection

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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, M8166 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 antimycotic 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

P913 Zinc: a modulator of influenza A virus induced programmed cell death

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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 adherant 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.

P914 Analysis of microRNA expression in cytological cervical samples and correlation with HPV infection

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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 analyzed 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 Biosistems). The expression levels of each miRNA was calculated by the $2^{-\Delta\Delta Ct}$ method against two different housekeeping snRNAs.
Results: Out of 40 samples, 10 were HPV-negative, 10 had low-risk HPVs 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-16 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.

P915 The pathogenesis of a novel enterovirus serotype EV-94

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Objectives: Enterovirus 94 (EV-94) is a recently described enterovirus serotype that is, together with EV-68 and EV-70, a member of Human enterovirus D species. EV94 was found during poliovirus surveillance from waste water in Egypt and from acute flaccid paralysis patients in the Democratic Republic of Congo. The seroprevalence studies in the Finnish population have revealed a high prevalence of EV-94 over past two decades. In this work, we studied the ability of EV-94, to infect, replicate and inflict damage in cellular models of tissues that are considered to play essential role in enterovirus induced diseases. These models include human leukocytic cell lines, human primary endothelial cells and human primary pancreatic islets.
Results: EV-94 was able to infect, form infectious progeny and inflict cell death in cellular models of tissues that are considered to be of importance in the pathogenesis of enteroviruses, including leukocyte lines with granulocytic, monocytic, B-cell or T-cell characteristics, human primary endothelial cells and pancreatic islets. Moreover this virus is able to inflict damage on pancreatic islet beta-cells.
Conclusion: These results provide in vitro evidence, that EV94 might be a potent pathogen and should be considered as a potentially diabetogenic enterovirus serotype.

P916 Prevalence of EBV and CMV in tonsils among patients with recurrent tonsillitis and tonsillar hypertrophy in Greece

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Objective: To compare the prevalence of EBV and CMV in tonsils between patients with recurrent tonsillitis and tonsillar hypertrophy.
Methods: A total of 194 patients, who underwent a total or partial tonsillectomy were included. The patients were categorised into two groups: a recurrent tonsillitis group (Group I, 105 patients) and a tonsillar hypertrophy group (Group II, 89 patients). The tonsillar tissues extracted during surgery were immediately transferred in sterile dry containers, and they were shipped to the laboratory immediately. Pieces of tonsillar tissue from each patient were chosen for simultaneous extraction of DNA, using the commercial kits (Invitrogen) according to manufacturers instructions. The detection of EBV and CMV was performed by Real-time PCR (Nanogen Advanced Diagnostics). The association between the two disease group (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.
Conclusions: The high prevalence of EBV among patients with recurrent tonsillitis and tonsillar hypertrophy verify that the tonsils are a potential reservoir for EBV infections.

P917 SEC14 is required for secretion of the fungal invasin, phospholipase B1, and virulence of the AIDS-related pathogen, *Cryptococcus neoformans*

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Objectives: 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 *Saccharomyces cerevisiae* and *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, *C. neoformans*.
Methods: The coding region of the putative SEC14 gene and two homologues, SFH1 and SFH5, were disrupted in *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, 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 *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)

[1] Chayakulkeeree et al. Mol Microbiol 2008;69:809–26.

P918 Antifungal activity of human beta-defensins 1, 2 and 3 against filamentous fungi

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Objectives: In the present study we demonstrate the potent antifungal properties of human beta defensins (hbD1, hbD2 & hbD3) against representative clinical isolates of filamentous fungi.
Methods: Defensins. Recombinant peptides of hbD1, hbD2 & hbD3 were purchased from Peprotech EC, UK. Lyophilised peptides were

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 by the lactophenol blue method and further validated by determining their carbon assimilation profiles using the ID32C system (Biomérieux, Marcy d'Étoile). Routine antifungal susceptibility testing (AFST) for itraconazole, fluconazole, voriconazole, 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 30C and resuspended in 10 mM sodium phosphate Buffer (PB) to obtain a final concentration of 10^6 cells/ml. Aliquots of diluted cells were exposed to varying concentrations of defensins for 24 h at 30C 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&2.
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.

P919 Formation of stomathopathies in correlation with presence of *Candida* and *Lactobacillus* strains

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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 (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. salivarius* 21%, *L. rhamnosus* 12%, *L. brevis* 10%, *L. acidophilus* 7%, *L. para paracasei* 7%, *L. cellobiosus* 5%, *L. plantarum* 2%, *L. delb. lactis* 2%, *Lc. 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. salivarius* 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.

P920 Mapping the host response to cerebral aspergillosis by integrative transcriptome analysis

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Objectives: Cerebral aspergillosis is fraught with a mortality exceeding 90%. Prerequisite for therapeutic advance is the knowledge of the pathophysiologic processes underlying the disease. Setting the global gene expression profile in the context of biological meta-data resources permits to unravel the complex pathological processes that determine the outcome of cerebral aspergillosis.
Methods: Gene expression was assessed in an infant rat model of experimental cerebral aspergillosis at 6 h, 72 h and 120 h after infection using the Affymetrix GeneChip® Rat Genome 230 2.0 arrays. The significance of transcriptomic changes was calculated with moderate Bayesian t-statistics using R. Data annotation was performed by software packages provided by the R based Bioconductor project.
Results: Of the 31,000 genes represented on the chip approximately 18,000 were expressed and detected on the chip in both control and infected animals. After statistical testing with the consideration of a false discovery rate (Benjamini-Hochberg $p \leqslant 0.05$), approx. 1550 genes were found to be significantly regulated at 72 h after infection and approx. 600 genes at 120 h after infection. According to data clustering and GO annotation most of the differentially expressed genes are related to the immune system and inflammatory host response. The findings validate gene microarray as an appropriate tool to decipher complex regulatory pathways in aspergillosis. Nevertheless, there were a considerable number of genes with unknown functions or not yet established connections to infection or neuronal tissue.
Conclusion: The results expand the current knowledge of pathways leading to neuronal injury in cerebral aspergillosis by integrating differential gene expression data in the context of biological research databases. This approach is a promising strategy to identify new targets for further study and, hopefully, therapy.

P921 Sero-prevalence of Rift Valley fever in south-western Saudi Arabia and study of risk factors

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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 Qunfuda regions (Southwest Of Saudi Arabia), potential risk factors leading to the infection and to elucidate the predisposing factors of developing severe RFV 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-Arda, Samtah, Beesh, Al-Birk, Al Gahma, Muhayeel, Al-Majardah and Al-Qunfuda) 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 outbreak. 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 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-specific IgG among the general population and the lack of recent RVF activity among humans in th study areas, the major risk factors were contact with animals especially aborted animals.

In vitro resistance of Gram-positive cocci

P922 Identification by genomic and genetic analysis of two new genes playing a key role in glycopeptide intermediate resistance

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Endogenous, low-level glycopeptide resistance in *Staphylococcus aureus* results from multi-factorial genetic changes. Comparative genomic hybridisation analysis revealed the specific deletion of a 1.8 kb segment, encompassing two adjacent open reading frames (ORFs) of unknown function, in a teicoplanin-susceptible revertant (strain 14–4rev) compared to its isogenic, teicoplanin-resistant parental strain 14–4. This provocative finding prompted us to perform a detailed genetic analysis of the contribution of this genomic segment to glycopeptide resistance. Despite repeated efforts in our laboratory, 14–4 and 14–4rev have proven refractory to most genetic manipulations. To circumvent this difficulty, we evaluated the contribution of both putative ORFs (designated as teicoplanin resistance factor trfA and trfB) on teicoplanin resistance in a different, genetically tractable background. Genetic analysis showed that single or double trfA and/or trfB mutations abolished teicoplanin resistance in two independent teicoplanin-resistant derivatives of NCTC8325 strain ISP794, generated by two-step passages with the drug. The frequency of teicoplanin-resistant mutants was markedly decreased by the absence of trfAB in the teicoplanin-susceptible ISP794 background. Nevertheless, a low rate of teicoplanin-resistant mutants was selected from ISPtrfAB, thus indicating an additional contribution of trfAB-independent pathways in emergence of low-level glycopeptide resistance. Further experiments performed with the clinical GISA isolate NRS3 indicated that trfAB mutation could affect not only teicoplanin, but also vancomycin and oxacillin resistance. In conclusion, our study demonstrates the key role of two novel loci in *S. aureus* endogenous, low-level glycopeptide resistance, whose precise molecular functions warrants further investigation.

P923 The regulatory mechanisms of macrolide-lincosamide-streptogramin B resistance in methicillin-resistant *Staphylococcus aureus*

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Objective: The cross resistance to macrolide, lincosamide and streptogramin B (MLS-B) in *Staphylococcus aureus*, depending on dimethylation of adenine A2058 in V domain of 23S rRNA, is mediated by the presence of the ermA, ermC, or rarely ermB or ermF methylases genes. The expression of the MLS-B resistance phenotype needs an induction or proceeds constitutively. The regulation of expression occurs on the level of initiation of translation. In the case of ermA, important role acts the ermAR fragment, located downstream of the regulated ermA gene, which can form a hairpin secondary structure. Mutations in ermAR can lead to constitutive phenotype. The aim of the work was the attempt to resolve mechanism of development of constitutively resistant mutants among strains harbouring inductive ermA gene.
Methods: 94 MRSA strains were examined for the inductive of resistance by disc diffusion method according to CLSI recommendations. In all these strains the presence of ermA, ermB, ermC and msrA/B genes was checked with PCR technique. To the further investigations strains with inducible phenotype of MLS-B resistance and possessing ermA as the only determinant of macrolide resistance were chosen. Spontaneous mutants constitutively resistant were obtained in the BHI-agar with 16 mg/l of lincomycin. Mutants were examined for the regulatory region ermAR, using PCR with primers surrounding 574 bp fragment.
Results: Among investigated MRSA, 21 strains with inducible MLS-B resistance and possessing ermA as the only determinant of macrolide resistance were found. The constitutive MLS-B resistant mutants were obtained from each of the parent isolate, with an average frequency 10–8. Several mutants of each parent strain (total number 93) were taken to the amplification of ermAR fragment. The obtained products were in wide range of size (500–2000 bp). The most frequent (59.1%) was 574 bp fragment, which was the same length as the wild product. The other products contained some 20- and 80-bp deletions (31.2%) and smaller (10-, 25-bp) or larger (1400 bp) insertions (9.7%). For several mutants more than one product was obtained.
Conclusions: The constitutive mechanism of MLS-B resistance may be procured as an effect of various mechanisms: point mutations or insertions, duplications or deletions some larger fragments of DNA. These changes prevent the ermAR cistron from forming the hairpin structure, which make the ermA RBS region constitutively available to the ribosome.

P924 A fluorescent multicolour multiplex real-time PCR protocol for the simultaneous detection of SCCmec types I to V in methicillin-resistant *Staphylococcus aureus*

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Objectives: MLST, Spa typing and MLVA are well established methods for molecular typing of *Staphylococcus aureus* isolates. Yet, these methods are restricted to targeting core genomic elements. It has become increasingly clear that mobile element typing is a valuable addition to the molecular epidemiology of *S. aureus* infections. The most valued mobile target for typing to date is the methicillin resistance gene-bearing staphylococcal chromosome cassette (SCCmec).
Methods: A multiplex real-time PCR was developed for identification of the abundant SCCmec types I to V. The assay was evaluated on 212 clinical methicillin-resistant *Staphylococcus aureus* isolates from various lineages.
Results: When comparing the assay with established SCCmec typing methods, the correct SCCmec type was identified in 210 of 212 (99%) isolates.
Conclusion: When combined with core genome typing, this real-time PCR assay provides a versatile means for SCCmec typing both on local outbreak scale, and for high throughput epidemiological studies.

P925 Molecular characteristic of *Staphylococcus aureus* associated with bacteraemia in Poland during 2005

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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 t084 (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

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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 Chromogenic MRSA Agar) 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.
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P927 Emergence of "animal" MRSA ST398 as coloniser and as infectious agent in humans

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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 famers 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 MRSAST398.
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* spp. Isolates in Blood Cultures from a Spanish site during a phase 3 clinical trial of topical administration of omiganan 1% gel

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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 $\geqslant$4 mg/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 pSCFS3 were used to PCR map the cfr surrounding region. Gene location was accessed by Southern blot and hybridisation. *S. aureus* isolate was submitted for spa and MLST typing.
Results: A 77 y/o 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 mupirocin, neomycin, ciprofloxacin, erythromycin, gentamicin and oxacillin. Both strains were resistant to chloramphenicol, clindamycin, linezolid, retapamulin 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 tnpB 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.

P929 Non-mutational cfr-mediated linezolid resistance *Staphylococcus epidermidis* isolates

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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. Macrorestriction 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 $\geqslant$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 methylase conferred full resistance to linezolid (MIC $\geqslant$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, here 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-mutational 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.

P930 A C2534T mutation in the 23S rRNA gene responsible for linezolid resistance in a clinical *Staphylococcus epidermidis* isolate

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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 disk diffusion test results, *S. epidermidis* was resistant to oxacillin, cefoxitin, tobramycin, gentamicin, ciprofloxacin, ofloxacin, fucidic acid, erythromycin and clindamycin, intermediate resistant to linezolid and susceptible to vancomycin, teicoplanin, tetracycline, daptomycin and tigecycline. The MICs to linezolid, oxacillin, 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. To confirm that this mutation involved to the linezolid expression, a total of 20 *S. epidermidis* isolates, genetically unrelated and linezolid susceptible were also tested for the presence of 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.

P931 Continuous increase of antibiotic resistance among non-vaccine type *Streptococcus pneumoniae* in the era of 7-valent conjugate pneumococcal vaccine

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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 ($0.12 \leqslant$MIC$\leqslant$1 ug/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

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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 91.1%. Penicillin 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%). Nonsusceptibility 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

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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 β-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

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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 phosphoglucosamine mutase GlmM involved in peptidoglycan biosynthesis, being implicated in β-lactams susceptibility. In order to further elucidate the association of StkP in *S. pneumoniae* in susceptibility to β-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 PBP 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, PBP2B and PBP1A 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 profiles 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 mef other than mef(A) in *Streptococcus pyogenes*

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Objectives: In *Streptococcus pyogenes*, erythromycin (ERY) resistance due to efflux is mainly associated with the mef(A) subclass of the mef gene, that is carried by a conjugative transposon/prophage. The aim of this study was to characterise the genetic elements carrying mef subclasses other than mef(A), in selected *S. pyogenes* isolates.
Methods: From a global collection of clinical isolates of *S. pyogenes*, 10 isolates carrying mef other than mef(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 mef(E), rarely reported in *S. pyogenes*, and tet(M). The 5 ERY-CHL resistant isolates carried mef(I), a mef subclass recently described, and catQ. The ERY-TET-CHL resistant isolate carried a novel subclass of mef, 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 mef(E)/tet(M)-positive isolates, mef(E) 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/tet(M)/catQ-positive isolate showed the same genetic structure of the 5216IQ complex with the exception of a ca. 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*.

P936 Tn1806, the erm(TR)-carrying genetic element of *Streptococcus pneumoniae*

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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 tetronasin efflux pumps and a spectinomycin fosfotransferase. Comparative nucleotide analysis confirmed similarity of Tn1806 with ICE10750 RD-2, the *Streptococcus pyogenes* erm(TR)-carrying element. Tn1806 is approximately 4 kb larger in size than ICE10750 RD-2 due to the presence of additional regions such as 3 ORFs found also in RD1, a chimeric element of *S. pyogenes* MGAS6180. 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 differing Tn1806/ICE10750 RD-2 from the *F. magna* element carried genes related to drug resistance such as those conferring resistance to erytromycin, tetronasin and spectinomycin in Tn1806 and ICE10750 RD-2 or a series of ABC multidrug resistance efflux pumps in *F. magna*.
Conclusions: 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.

P937 Tigecycline activity against macrolide resistance in *Streptococcus pneumoniae* mediated by mefE and ermB determinants: a global analysis 2008

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Background: Macrolide resistance in *S. pneumoniae* (MRSP) has increased steadily in the last decade. Two important determinants in MRSP are the mefE (clindamycin-resistant, macrolide-resistant) and ermB (clindamycin-resistant, macrolide-resistant) genes. This report evaluates tigecycline and comparator drug activity from a global population of MRSP in clinical isolates collected in the T.E.S.T. program during the years 2004 to 2008.
Methods: 1,962 *S. pneumoniae* clinical isolates were collected from 163 investigative sites in 31 countries and tested for erythromycin resistance. 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 a reference laboratory using broth microdilution panels and interpreted according to CLSI guidelines.
Results: Summary data for the 557/1962 (28.4%) MRSP strains categorised by ermB and mefE determinants 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* with ermB determinants and was equivalent to clindamycin against mefE determinants. Tigecycline in vitro activity suggests that tigecycline may be effective against this important clinical pathogen and resistant phenotype.

Drug	*ermB* (n = 254)				*mefE* (n = 244)			
	MIC_{50}	MIC_{90}	% Sus	% Res	MIC_{50}	MIC_{90}	% Sus	% Res
Tigecycline	0.03	0.12	na	na	0.03	0.12	na	na
AmoxClav	0.5	4	71.3	9.8	0.25	2	92.2	1.6
Cefdinir	>2	>2	39.8	59.1	1	>2	46.3	46.3
Ceftriaxone	0.5	1	87.8	3.5	0.25	1	92.2	1.2
Cefuroxime	4	8	38.2	59.1	1	8	48	49.6
Clindamycin	>64	>64	0	100	0.06	0.12	100	0
Erythromycin	>64	>64	0	100	16	64	0	100
Levofloxacin	0.5	1	99.2	0.4	0.5	1	99.2	0.8
Linezolid	=0.5	1	100	0	=0.5	1	100	0
Minocycline	4	8	3.9	1.6	=0.25	4	5.7	0.4
Penicillin	1	4	23.6	40.9	0.25	2	27	25.4
Trimeth Sulfa	4	>4	27.2	59.1	4	>4	30.3	54.1

P938 In vitro activity of garenoxacin against clinical isolates of levofloxacin-resistant *Streptococcus pneumoniae* with amino acid substitutions in QRDRs of gyrase and topoisomerase IV

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Objectives: Fluoroquinolone-resistant *S. pneumoniae* are increasing rapidly throughout the world. In vitro activities of garenoxacin (GRN), a des-fluoro (6)-quinolone, were evaluated against clinical isolates of *S. pneumoniae* resistant to levofloxacin (LVX), characterised genetically.
Methods: Quinolone resistant-determining regions (QRDR) of gyrA, gyrB, parC and parE of 19 LVX-resistant strains, isolated from adult patients in Japan, were analyzed by PCR-based sequencing. MICs of GRN and other fluoroquinolones were determined by a microdilution broth method. The 50% inhibitory concentrations (IC50 s) against supercoiling activity of DNA gyrase and decatenation activity of topoisomerase IV (Topo IV) of LVX-resistant *S. pneumoniae* D-3668 (GyrA: S81F, ParC: S79F, GyrB, ParE: no mutation) were measured referring to previously described methods.
Results: MICs of GRN against LVX-resistant *S. pneumoniae* (LVX MIC range, 8–32 mg/L) were 0.125 to 1 mg/L, 16- to 256-fold lower than those of LVX, and 4- to 16-fold lower than those of moxifloxacin (MXF, MIC range, 0.5–8 mg/L). Seventeen of 19 strains had single or double amino acid (Aa) substitutions in the QRDR of GyrA, which included D80A, S81Y/F, E85G and S114G. In GyrB, 2 of 19 strains had a single Aa substitution (D435N/E or E474K). Fourteen of 19 strains had single or double Aa substitutions in ParC, which included S79Y/F, D83N/Y, N91D and K137N. In ParE, 14 of 19 strains had single or double Aa substitutions (D435N, I460V and E474K). Even if there was a mutation of S81Y/F in GyrA, GRN showed potent antibacterial activity if ParC did not have a mutation such as S79Y/F. The IC50 s of GRN against

supercoiling activity of DNA gyrase and decatenation activity of Topo IV of LVX-resistant *S. pneumoniae* D-3668 were 357 mg/L and 3.76 mg/L, respectively. GRN showed 3.6- and 1.7-fold (gyrase), 11.0- and 2.7-fold (Topo IV) more potent inhibitory activity against the two target enzymes than of LVX and MXF, respectively.
Conclusions: GRN showed more potent in vitro antibacterial activity against LVX-resistant *S. pneumoniae* than MXF. The potent activity of GRN against the LVX-resistant strain was based on potent inhibitory activity against the target enzymes.

P939 Epidemiological analysis of pneumococcal carriage in Hungarian children from a day-care centre

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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 on the carriage. As this vaccine became widely available in Hungary 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.

P940 Analysis of alterations in the leader sequence of the erm(B) gene of different species of viridans group streptococci expressing MLSB resistance phenotype

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Objectives: The main methylase conferring MLSB resistance among viridans group streptococci (VGS) is encoded by the erm (B) gene. To determine the sequence of the erm (B) regulatory region, we analyzed the leader sequence of the gene in different VGS species and in *S. bovis* showing cMLSB and iMLSB phenotypes. A telithromycin-resistant *S. bovis* isolate was also included.
Methods: From 1998 to 2006 223 VGS and *S. bovis* 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. costellatus*, 2 *S. bovis*, and 1 *S. salivarius* cMLSB phenotype, 1 *S. bovis* iMLSB and 1 *S. bovis* cMLSB showing telithomycin 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. bovis* cMLSB isolates resulting in A25T substitution and in the telithromycin-resistant *S. bovis* 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) was also observed in the telithromicin resistant *S. bovis* 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. bovis* strain and could be implicated in the ketolide resistance.

P941 Rapid decrease in the prevalence of macrolide-resistant group A streptococci in Spain

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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.
Introduction: 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 inversion 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%).

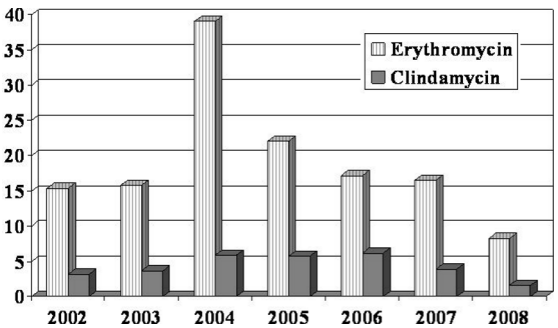

Figure 1. Resistance to macrolides and lincosamides in *Streptococcus pyogenes*.

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)

[1] Oliver MA, García-Delafuente C, Cano ME, Pérez-Hernández F, Martínez-Martínez L, Albertí S. Rapid decrease in the prevalence of macrolide-resistant group A streptococci due to the appearance of two epidemic clones in Cantabria (Spain). J Antimicrob Chemother. 2007 Aug;60(2):450–2. Epub 2007 May 31.

P942 Bacteriophage-mediated transfer of erythromycin and tetracycline resistance genes among group A streptococci

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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" lysogenic 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 0.2 mg/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 erythromycin for the selection of ERY-resistant lysogenic clones.
PCR was used to check for the emm-type, mefA, and tetO genes before and after lysogenic transfer experiments, and to detect phi-m46 DNA in the supernatant of induced donor cultures.
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 phage-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.

P943 Amoxicillin resistance of oral streptococci in healthy patients undergoing tooth extraction

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Background: Reduced susceptibility to penicillin is commonly observed in oral streptococci from patients exposed to recurrent courses of antibiotics, but little is known about the susceptibility to penicillins of the commensal oral flora in healthy adults.
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 sanguinis*, *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.
Conclusion: 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.

P944 Rapid detection of G2576T mutation conferring linezolid resistance in enterococci

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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 this SNP correlates with the level of resistance to linezolid. A rapid test based on Allele Specific PCR (AS-PCR) was designed to detect the SNP at position 2576 of the 23S rRNA genes of *Enterococcus*.
Methods: The assay was evaluated on 52 enterococcal isolates (*Enterococcus faecalis* (n = 40) or *Enterococcus faecium* (n = 12)) and 4 non enterococcal isolates. Thirty isolates were susceptible to linezolid (MIC of linezolid <4 mg/L); 26 isolates were linezolid resistant (MIC of linezolid >4 mg/L) known to contain by sequencing analysis either the G2576T SNP (n = 23) or the G2505A SNP (n = 3).
Real time PCR was performed using a specific primer in which the second nucleotide from the 3′ end was designed to match site of the 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-rime AS-PCR assay for detection of G2576T mutation in *Enterococcus* allows 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 witch 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

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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 14 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 domain V of the 23S rRNA subunit, to detect mutations on the peptidyl-transferase centre of the bacterial ribosome. Sequencing of the PCR 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 MIC's 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 linezolid MICs. PFGE revealed multiple unrelated electrophoretic patterns indicating a multiclonal outbreak. Resistance was correlated with prolonged linezolid treatment in 11 patients, while three patients had not received linezolid.
Conclusions: Both *E. faecium* and faecalis have developed linezolid-resistance in the ICU of our hospital. Most of the isolates belonged to unrelated clones, indicating that linezolid resistance was not related to patient-to-patient transmission and is probably endemic in our institution. Rational use of linezolid and intensive infection control measures are required to restrict further isolations of these difficult to treat pathogens.

P946 Clonal spread of penicillin-resistant ampicillin-susceptible *Enterococcus faecalis* among bloodstream infections in Denmark

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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 nitrocefin disks to test for β-lactamase activity (Oxoid, UK). A total of 20 PRASEF isolates and 13 random penicillin-susceptible isolates were genotyped by gdh sequence analysis, and isolates displaying allele type 12 were further analyzed by multi-locus sequence typing (MLST). Isolates belonging to the predominant sequence type (ST) were characterised by pulsed-field gel electrophoresis (PFGE).
Results: We recorded 20 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.

Origins, resistance phenotypes and genotypes of 20 penicillin-resistant ampicillin-susceptible *Enterococcus faecalis* (PRASEF) isolates from blood cultures in North Denmark Region.

Strain ID	Site of infection	Origin[a]	Hospital	MIC (μg/ml)					Sequence type		PFGE
				AMP	PEN	IPM	GEN	VAN	*gdh*	MLST	
B63792	Urinary tract	NH	8	1.5	>32	6	>1024	4	12	6	A1
B37814	Undetermined	N	8	1.5	>32	8	>1024	3	12	6	A1
B71580	Hepato-biliary-pancreatic tract	HC	8	1.5	>32	8	>1024	4	12	6	A1
B33621	Urinary tract	N	7	1	16	8	1024	3	12	6	A1
B135456	Urinary tract	N	6	4	>32	8	96	2	12	6	A1
B69486	Aortic vascular prosthesis	C	4	1.5	>32	6	>1024	3	12	6	A1
B15725	Undetermined	N	8	1.5	32	32	>1024	3	12	6	A2
B98380	Undetermined	C	1	3	16	4	>1024	2	12	6	A2
B37093	Undetermined	N	8	1	>32	4	>1024	3	12	6	A3
B72715	Urinary tract	N	4	2	>32	6	1024	3	12	6	A3
B54027	IV catheter	N	8	3	>32	6	>1024	3	12	6	A4
B147794	Diabetic gangrene	N	8	0.75	>32	4	>1024	2	12	6	A4
B33672	Perirenal abscess	N	3	1	32	4	1024	3	12	6	A4
B110274	Undetermined	N	8	3	>32	4	>1024	3	12	6	A5
B85040	Urinary tract	HC	8	1.5	16	4	>1024	3	12	6	A6
B67010	Endocarditis	N	8	1.5	>32	8	>1024	3	12	6	A7
B37957	Surgical site infection	N	8	1.5	>32	6	>1024	3	12	6	A8
B84847	Hepato-biliary-pancreatic tract	N	5	1.5	>32	2	16	3	2	23	NT[b]
B56765	Undetermined	N	8	4	>32	32	>1024	4	4	28	NT[b]
B16457	Undetermined	N	8	4	32	8	96	4	New	New	NT[b]

[a] N, nosocomial; C, community-acquired; HC, health care-related.
[b] NT, not typed by pulsed-field gel electrophoresis (PFGE).

P947 Spread of EfcTn1 in enterococci from different ecological niches

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Objectives: Conjugative 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 HiRECC 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 relaxases of CTn from Gram positives (EfcTn1, Tn916, Tn5397, Tn5398, CW459TetM or Tn5386) associated with resistance to tetracycline and/or erythromycin. Positive samples for int/xisEfCtn1 were amplified with tetSFW-IntRVEfcTn1 and orf20 Tn916/orf14 Tn916/orf13Tn916-tetSRV (long-PCRs). PCR products were digested and RFLP patterns were compared (TaqaI/AluI). One representative *E. faecalis* (Efc) was partially

sequenced. Antibiotic susceptibility test was done by disc diffusion/agar dilution and clonality by PFGE.
Results: int/xisEfCtn1 were detected in 7 Efc (5-HH; 2-USA CS) and in 2 Efm (same clone; 2 piggeries). All but 2 (HH-TeI) were TeR. tetS, tetM and tetL were amplified in all, 6 and 1 isolates, respectively. RFLP profile of tetSFW-IntRVEfcTn1 (~4 kb) was the same in all isolates. Sequencing of one Efc revealed the organisation previously described to EfcTn1 with int/xisEfCtn1 followed by 4 orf showing >50% of aminoacidic similarity with orf8, orf7, orf9, orf6 of Tn916 and tetS. Amplification of Tn916 sequences as orf13, orf14 or orf20 (relaxase) and tetSRV was negative. Seven of the enterococci also harboured Tn916 (n = 5), Tn917 (n = 4), Tn5398 (n = 4) or CW459tetM (n = 1). Transconjugants contained Tns other than EfcTn1 supporting the low frequency rate of transposition previously described.
Conclusions: EfcTn1 is described for the first time in different ecological niches and seem to be more widespread than initially expected. Isolates from different settings showed a similar structure between tetS and the integrase suggesting genetic stability in this area.

P948 Portuguese healthy human volunteers are reservoirs of conjugative transposons associated with tetracycline and/or erythromycin resistance

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Objectives: Conjugative transposons (CTn) have contributed to the spread of tetracycline (Te) and erythromycin (Er) 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 *Enterococcus* spp-Ep) 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, tetL, tetS, tetK, ermB). The presence of known Gram positive CTn and other Tn frequently associated with them was screened by a multiplex PCRs for detecting integrases, excisionases, transposases and relaxases of Tn916, Tn917, Tn5397, Tn5398, EfcTn1, CW459TetM, or Tn5386.
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/xisTn916, tnpA/tnpRTn917, orf298Tn5398 or int/xisEfCtn1 was higher for Efc than for Efm or Ep (69%/25%/46%; 15%/8%/0%; 52%/25%/46%; 8%/0%/0% respectively). tndXTn5397 was more common among Efm (18%) than Efc (2%). IntCW459tetM 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% two or more of these genetic elements (int/xisTn916+orf298Tn5398–24%; int/xisTn916+tnpA/tnpRTn917–6%; int/xisTn916+tnpA/tnpRTn917+orf298Tn5398–3%; int/xisTn916+tnpA/tnpRTn917+orf298Tn5398+int/xisEfCtn1–2%; 8 other combinations with 1 isolate each).
Conclusion: Portuguese HV are important reservoirs of Gram positive Tns associated with Te and Er resistance. Some *Clostridium* sp typical genetic elements were isolated for the first time in enterococci from this setting (CW459tetM, Tn5398) and their high occurrence suggest frequent mobilisation among different bacterial genera.

P949 Characterisation of Gram-positive conjugative transposons among international epidemic vancomycin-resistant isolates of *Enterococcus faecalis* and *Enterococcus faecium*

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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* (Efm) and 32 *E. faecalis* (Efc) 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 Efc (CC2, CC9, CC87) and Efm (CC17). All but two isolates (Efm) were resistant to vancomycin (vanA-68%; vanB-25%, vanG-1%). Characterisation of tet (tetM, tetO, tetL, tetS, tetK) and ermB genes was performed by PCR. The presence of integrases/excisionases from Gram positive CTns or other Tn associated to them (Tn916, Tn917 Tn5397, Tn5398, EfcTn1, 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 Efc (69%, 41%, 9%) and Efm (38%, 21%, 0%). ermB was also detected in Efc (100%) and Efm (87%). Occurrence of int/xisTn916/Tn1545, orf298Tn5398, tnpA/tnpRTn917 was higher among Efc [47%-10 countries (c), 72%-6c, 44%-6c] than Efm (13%-7c, 18%-6c, 13%-4c). Conversely, IntCW459tetM was more common among Efm than Efc (30%-8c and 19%-4c, respectively). tndXTn5397 was only detected in 2 Efm and 1 Efc from Spain and Portugal and int/xisEfCtn1 in two Efc 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 HiRECC strains in the hospital setting. This is the first description of IntCW459tetM in enterococci, and of int/xisEfCtn1 among enterococcal strains associated with the hospital environment.

P950 Prospective study of Corynebacteria other than *Corynebacterium diphtheriae* in clinical specimens: identification, clinical relevance and antibiotic susceptibility

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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. macginleyi* 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 immunocompromised and the sex ratio was of 0.4.
Of the 33 strains, 8 (24.2%) came from ear infection specimens, 6 (18%) from biomaterials essentially from thorax drains (3/6) and 6 (18%) from wound 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. jeikeium* and *C. pseudodiphtericum*.

Conclusion: Corynebacteria other than *Corynebacterium diphtheriae* are a non 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

P951 Management of infective endocarditis: clinical experience with outpatient parenteral antibiotic therapy

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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, endothelial 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 reported. Analyses were performed using Stata (version 10.1, College Station, TX).

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 $\geqslant$ 4 weeks of therapy, with $\geqslant$ 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 endothelial surface, requirement for surgery, total treatment duration and OPAT

Infecting organism	Number of patients (N = 43)	% Left-sided disease (N = 33)	% Required emergent surgery (N = 12)	Duration of treatment (weeks)	% OPAT
Viridans streptococci	8	(7/8) 88	(3/8) 38	$\geqslant$4	72
Enterococcus faecalis	5	(5/5) 100	(2/5) 40	$\geqslant$6	80
Methicillin susceptible *Staphylococcus aureus*	9	(5/9) 56	(1/9) 11	$\geqslant$4	68[a]
Methicillin resistant *Staphylococcus aureus*	1	(0/1) 0	(0/1) 0	6	90
Staphylococcus non *aureus*	5	(4/5) 80	(2/5) 40	$\geqslant$5	80
Culture negative	9	(7/9) 78	(2/9) 22	$\geqslant$5	74
Other streptococci	4	(3/4) 75	(1/4) 25	$\geqslant$4	75
HACEK	1	(1/1) 100	(1/1) 100	4	68
Proteus mirabilis	1	(1/1) 100	(0/1) 0	6	66

[a] 1 patient lost to follow-up.

P952 Outcome of empiric versus targeted antibiotic therapy in infective endocarditis, Medellin, Colombia. Study of 120 cases over a 20-year period

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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 microbiologic 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 EI 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.

P953 Epidemiology, clinical and microbiological features of infectious endocarditis: a review of 54 cases

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Objectives: The purpose of the study was to investigate the aetiology, 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 transoesophageal (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

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Objectives: To know the clinical features and possible aetiologic agents of the blood culture negative endocarditis (BCNE).

Methods: Descriptive cross sectional study in a serie 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 symptons until diagnosis were 68±126 days. The symptons 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 germen, in 19 (15.8%) *Coxiella burnettii*, in 4 (3.3%) *Brucella*, in 4 (3.3%) Fungus, 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. burnettii* 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) Negatives blood cultures endocarditis presents a high surgery percentage with similar mortality than positives blood culture endocarditis.

P955 Brucellosis as an aetiology for culture-negative endocarditis in endemic regions

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Background: Brucellosis is an orally transmitted bacterial through unpasteurised milk products manifesting with fever, chills, bone pain, fatigue and myalgia. Although it has been eradicated in some countries, it is still endemic in other regions. If left untreated or partially treated, complications may occur. Endocarditis is the most fatal complication. In this study we evaluated brucellosis as an aetiology for endocarditis in Iran as an endemic region.

Method: 572 cases defined as endocarditis (Duke Criteria) (1991–2008) in 3 referral heart surgery centres were retrospectively evaluated. 365 out of 577 were culture negative based on routine culture media after 48 hrs. 11 (3%) culture negative cases were proved as brucellosis by serology or blood culture after 2–3 wks. Patients were followed for clinical, serological findings and outcomes as measures.

Results: Brucellosis was the aetiology for endocarditis in 1.9% totally and 3% in culture negative cases. Fever (100%), Chills (90.9%) for more than one year were the most common complaint. Aortic (54.5%) and Mitral valve (36.3%) were the most commonly involved valves. Valsalva sinus was involved in one case. Operation in addition to antibiotic therapy was performed in 10 cases out of 11 (90.9%). The complications observed in these patients are renal failure, back pain, arthritis and CNS involvement. Blood culture was positive in 4 patients (36.36%). 6 patients survived and 5 patient died before, during or early after surgery. 2 patient developed brucella prosthetic valve endocarditis 2 years after surgery. Aortic root aneurysm was observed in one case as a complication for brucella endocarditis 16 years after primary surgical treatment.

Conclusion: Brucellosis should be considered in culture negative endocarditis in endemic regions. It is associated with high mortality (45%). Serologic and specific culture media for isolating the organism should be ordered in case.

P956 Infective endocarditis: Czech experience. Results of a multi-centre incidence study

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Objectives: Infective endocarditis (IE) seems to be changing and transforming into civilisation disease. We were interested in characteristics of the disease in our central European postcommunistic country.

Methods: A multicentre prospective observational study was carried out in 29 regional hospitals. The hospitals take care of 3,934,000 inhabitants what is 37.7% of the whole population of the Czech Republic. All the IE cases that occurred in the particular regions within one year period (February 2007–January 2008) were reported.

Results: In the monitored period, 124 cases of IE were reported, this implies the average annual incidence of 3.15 cases per 100 000 inhabitants. The patient's age range was 19–98 yrs; median 63 yrs. Men/women ratio was 91:33. One hundred and seven patients fulfilled criteria of "definite IE" according to the revised Duke criteria (Li, 2000). Only 74 pts (60%) were admitted in departments of cardiology/internal medicine initially. The others were admitted in ID departments (16 pts, 13%), neurology (15 pts, 12%), surgery (6 pts, 5%), or another departments.

There were 18 prosthetic valve endocarditis and 16 pacemaker endocarditis. Seven patients were intravenous drug addicts. Aortic valve was involved in 49 pts (40%), mitral valve in 43 pts (35%), both aortic + mitral in 6 pts (5%), tricuspid in 10 pts (8%). Other localisation or multiple site involvement was found in 14 pts (12%). Aetiological agent was *Staphylococcus aureus* in 36 pts (29%), coagulase-negative staphylococci 13 pts (10%), streptococci in 17 pts (13%), enterococci in 10 pts (8%), other bacteria in 8 pts (7%). No fungal agent was found. The aetiology was not established in 40 pts (32%); twenty-six of them were given antibiotics before collecting blood cultures.

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.

P957 The incidence of sepsis in a large Dutch University hospital: CRP and the SIRS criteria

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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 $\geqslant 2$ 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 without infection were identified.
Respiratory rate was not measured in 1973 episodes and was therefore not evaluated.
In patients without organ failure CRP gave an AUC of only 0.558. No additional differential effect was observed with addition of any of the SIRS criteria.
In patients with organ failure CRP combined with temperature yielded an AUC of 0.601.
In patients with shock CRP with any of the SIRS criteria did not significantly differentiate between septic shock and shock patients.
Conclusion: Temperature, heart rate, white blood cell count and CRP did not discriminate sufficiently between sepsis patients and comparatively ill patients without infection.

P958 The effect of bacteraemia on the immune response and outcome of patients with severe sepsis

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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 bacteraemia.
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 bacteraemia (group B, n = 17) and non-bacteraemic patients (group NB, n = 39). Severe sepsis was defined as the presence of confirmed infection and $\geqslant 2$ of the following criteria: (a) a temperature >38ºC or <36ºC, (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 1L-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, 29.4%). 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 bacteraemia and 10 out of 45 (22.2%) patients without bacteraemia died (difference not significant). Group B had similar levels of IL-6 with group A (121.3±32.9 vs 108.4.4±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-β (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 (14.8±2.4 vs 15.2±1.7, p = 0.8). Patients with bacteraemia 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 bacteraemia do not seem to have a different immune profile than patients without bacteraemia. Bacteraemia seems to influence LOS but not the final outcome.

P959 Analysis of immune competent cells following major and minor visceral surgery provides insight into origin of postoperative sepsis

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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. 24 h 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 analysed 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

48 hours postoperatively ($p < 0.005$) and even more 72 h postoperatively ($p < 0.0005$).
Conclusion: We describe a direct correlation between dimension of previous surgical trauma and immune dysfunction in the postoperative course. Major, especially protracted surgery results in significant suppression of immune function when compared to minor surgery. Consequently the incidence of postoperative severe sepsis is presumably increased following major compared to minor surgical procedure, which has to be considered right from the planning stage of extensive operations.

P960 Prognostic factors of community-acquired severe sepsis and septic shock

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Objective: We sought to determine the independent risk factors on mortality in community acquired bacteraemic 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 bacteraemic severe sepsis and septic shock were identified. Clinical, microbiologic and laboratory parameters were compared between hospital survivors and hospital deaths.
Results: The global mortality rate was 50%, 52.8% in septic shock and 41.2% in severe sepsis. One o 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 independently 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.

P961 Risk factors for and prognosis of sepsis secondary to severe or complicated pyelonephritis

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Objectives: The aim was to identify the possible risk factors associated with and 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 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±20.0 years (range, 14–97 years). Five hundred and eighty eight patients had one or more structural or functional urinary tract disorders. In multvariate analysis, male sex (OR: 1.03; CI95%: 1.02–1.04), recent urinary instrumentation (OR: 3.64; CI95%: 1.74–7.63), nosocomial acquisition, thrombocytopenia (OR: 2.57; CI95%: 1.66–3.96), creatinine (OR: 1.41; CI95%: 1.21–1.66), age (OR: 1.66; CI95%: 1.15–2.39), positive blood cultures (OR: 6.34; CI95%: 3.6–11), and grade III/IV sonographic ectasia (OR: 1.94; CI95%: 1.38–3.06) were associated to severe sepsis.
Mortality was 19.4% and 1.3% in patients with and without severe sepsis $p < 0.0001$.
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.

P962 *Streptococcus milleri* group sepsis: abdominal infections and macrolide resistance

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Objectives: We analyzed *S. milleri* group (*S. intermedius*, *S. anginosus* and *S. constellatus*) sepsis/bacteraemia cases in terms of clinical presentations and antimicrobial susceptibilities in Japan between January 2004 and March 2008. This analysis focused on the gastrointestinal and hapatobiliary system as a portal of entry of *S. milleri* group. Whereas *S. milleri* group is considered to be pathogens mainly in respiratory infections, the pathogenesis of *S. milleri* bacteraemia is not fully understood.
Methods: Clinical and demographic data were obtained from 15 patients in our 1300-bed tertiary hospital. Antimicrobial susceptibility testing was based on the CLSI criteria. Macrolide/lincomycin resistance related genes, ermB, ermTR, mefA and linB, were analyzed with PCR.
Results: Fifteen patients (aged 3–91 [mean 60 years]) had male preponderance (male: female = 12:3). As underlying conditions, 7 (46.7%) had hepatobiliary or digestive diseases such as cholangiocarcinoma, hepaticolithiasis, and 5 (33.3%) respiratory diseases including lung carcinoma, and 5 (33.3%) receiving chemotherapies against malignancy. As associated sites of infection, 5 (33.3%) were hepatobiliary, 4 (26.7%) gastrointestinal tract, 4 (26.7%) lung, respectively. In susceptibility testing, 5 isolates (33.3%) were resistant to erythromycin, and 2 (13.3%) to clindamycin. ErmB gene and mefA gene were detected in 2 (13.3%) and 3 (20.0%) isolates, respectively. None of the patients, however, had a history of long-term administration of macrolides or clindamycin.
Conclusion: The pathogenesis of *S. milleri* bacteraemia implicated retrograde bile duct infection and bacterial translocation from the gut to peritoneum. Macrolide-resistant *S. milleri* can cause bacteraemia in patients with no prior long-term macrolide use.

P963 A circulating factor of patients with septic shock stimulates release of angiopoietin-2 by human monocytes

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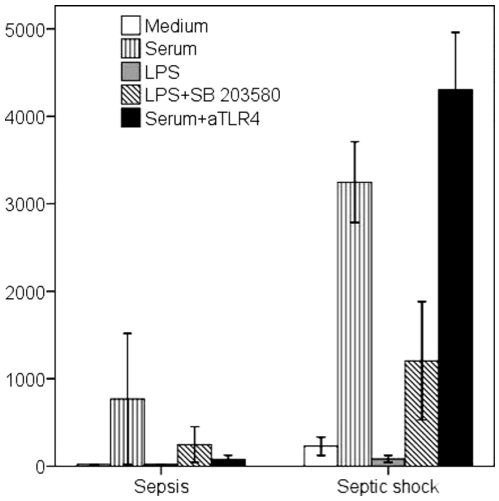
Objectives: Angiopoietin (Ang)-2 was shown to circulate in high levels in the serum of patients with septic shock (Orfanos S, et al. Crit Care Med 2007; 35: 199). The present study attempted to unravel the existence of any factor in serum stimulating the production of Ang-2 by human monocytes.
Methods: 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 10 ng/ml of lipopolysaccharide (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 10 ng/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

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Objectives: The BBL Pneumoslide is an agglutination test for the rapid identification of *Streptococcus pneumoniae*, consisting of latex beads coated with polyvalent antisera 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

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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

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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 48 h 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 sensibility and specificity of a score >6 was 21.4% and 95%, respectively.

Conclusions: Residence in a nursing home is associated with HCAB 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

P967 Spanish nationwide study on *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (GEIH-BLEE 2006). Emergence of CTX-M-15

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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 (2 mg/l) or ampicillin (100 mg/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 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: 0% (imipenem, meropenem), 2% (amikacin), 5% (ertapenem), 9% (tigecyclcine), 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.

P968 Diversity of extended-spectrum β-lactamases in *Escherichia coli*: second nationwide study in Spain (GEIH-BLEE 2006)

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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 (bioMerieux). 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.

P969 Emergence of *Escherichia coli* producing CTX-M-15 in Seville: epidemiological and clinical features

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Objectives: *Escherichia coli* clonal group ST 131 producing CTX-M-15 has emerged worldwide. Until recently, CTX-M-14 was the predominant enzyme produced by extended-spectrum β-lactamase-producing *E. coli* (ESBLEC) in Spain. We detected the emergence of CTX-M-15-producing isolates in our area (North of Seville, Spain) in 2006. We describe the clinical and epidemiological features of this cases in comparison with those producing CTX-M-14.
Methods: Prospective cohort of patients colonised or infected by ESBLEC in our area from September 2006 to March 2007. Epidemiological and clinical data were collected from all patients. We compared the features of patients with CTX-M-14 and CTX-M-15-producing isolates using the chi squared or the Mann-Whitney U tests. ESBL production was studied by PCR and sequencing; clonal relationship was studied by PFGE.
Results: There were 79 cases of colonisation/infection caused by ESBLEC during the study period. The ESBL was CTX-M-15 in 17 (21%) (data were available in 16) and CTX-M-14 in 24 (30%). Among CTX-M-15 producers, acquisition was nosocomial in 8 (50%), healthcare-associated in 2 (12%) and strict community in 6 (37%); 11 patients had urinary tract infections (68%), and 4 patients (25%) were bacteraemic. Although 11 patients had clonally related isolates, we could not find evident epidemiological link between them, except for 2 patients who were admitted to the same ward. Nosocomial acquisition was more frequent in CTX-M-15 producers than in CTX-M-14 (50% vs 21%, $p = 0.05$); also, COPD and haematologic cancer were somehow more frequent, although not significantly (31% vs 8%, $p = 0.09$). We found no other differences in predisposing factors or clinical features.
Conclusions: We describe the emergence of clonally-related CTX-M-15-producing *E. coli* in Seville. Although acquisition of these isolates was more frequently nosocomial than CTX-M-14-producers, there was no clear evidence of nosocomial transmission in most cases. Patients with these isolates more frequently had some underlying conditions and nosocomial infections.

P970 Emergence of CTX-M-15-producing *Escherichia coli* isolates in Seville, Spain

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Introduction: Recently, a virulent ciprofloxacin-resistant CTX-M-15-producing *E. coli* clone (O25b:H4-ST131) has globally disseminated.

This clonal group belongs to phylogenetic B2 group and it shares several plasmidic multirresistant 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-Marh 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 phylogroups were assigned by mutiplex 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 were resistant to ciprofloxacin, 74% to co-trimoxazole, and 79% to tobramycin. Twelve (57%) strains belonged 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. Four of six strains belonged to phylogenetic A group were included in the same cluster by PFGE (2 clinical isolates and 2 food isolates).

Conclusions: The increase of CTX-M-15-producing *E. coli* isolates observed recently in our area is mainly due to clone O25b:H4-ST131. Furthermore, we also detected spread of this enzyme among clonal *E. coli* isolates of phylogenetic group A from both food producing animals and humans.

P971 Outbreak of *Klebsiella oxytoca* strains harbouring blaCTX-M-15, blaOXA-1, qnrS1 and two copies of aac(6′)-Ib-cr genes in a paediatric intensive care unit

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Objective: To analyse an outbreak of broad-spectrum cephalosporin-resistant *K. oxytoca* strains in a paediatric intensive care unit and 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, ceftazidime, nalidixic acid, ciprofloxacin, levofloxacin, gentamicin, amikacin and tobramycin were of ⩾64, ⩾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 ISEcp1 and orf477. 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(3′)-II-IS26-aac(6′)-Ib-cr-blaOXA-1 structure, and the other one in the aac(6′)-Ib-cr-IS26-intI1 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: This report emphasizes 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.

P972 Shift in ESBL-types produced by Enterobacteriaceae in a Portuguese hospital

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Objectives: 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 1486 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 = 2), *E. asburiae* (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 (15/81, 19%) from *E. coli*, *K. pneumoniae*, *K. oxytoca* and *E. cloacae*. Both ESBL-types were consistently recovered during the studied period from the Medicine Service, although they were also detected in other hospital wards. All CTX-M-15-producers harboured blaOXA-1 and/or blaTEM-1/-116.

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.

P973 The molecular epidemiology of the ESBL enzyme, CTX-M in Enterobacteriaceae surveyed in north London

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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-M1, 2, 8, 9, 25 based on sequence homology. In the UK, over 90% of ESBLs have been sown 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, 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-M3-like alleles and 355 bp indicated CTX-M14-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 practises, particularly when investigating an outbreak. Adaptation of this rapid molecular method in 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 1. Prevalence of CTX-M expressing ESBL organisms

Strain	No. of strains (%)	CTX-M +ve			Total (%)
		M_{14} (%)	M_{15} (%)	$M_{15/28}$ (%)	
E. coli	124 (62.0)	3 (2.4)	12 (9.7)	4 (3.2)	19 (15.3)
K. pneumoniae	29 (14.5)	1 (3.45)	4 (13.8)	1 (3.45)	6 (20.7)
E. cloacae	10 (5.0)	0 (0)	1 (10.0)	1 (10.0)	2 (20.0)
Others	37 (18.5)	1 (2.7)	1 (2.7)	0 (0)	2 (5.4)
Total	200	5 (2.5)	18 (9.0)	6 (3.0)	29 (14.5)

P974 Epidemiology of ESBL-producing Enterobacteriaceae in Belgian hospitals: results of nationwide study in 2008

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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 synergy test and in the presence of boronic acid 400 µg disks for AmpC hyperproducers. ESBLs were characterised by PCR-sequencing assay targeting the blaTEM, blaSHV, blaOXA and blaCTX-M. Genes encoding the major plasmid-mediated AmpC β-lactamases and carbapenemases were also sought by PCR-sequencing.

Results: 91 hospitals (43 in Flanders, 31 in Wallonia and 17 in Brussels) sent 733 isolates (401 confirmed as ESBLE) originating from patients (mean age 71 years; range 0–98 yrs) hospitalised in medical (55%), surgical units (21%) or ICU (20%). ESBLE were mainly isolated from urinary (53%) and respiratory tract (20%), wound swabs (12%) and blood (4%). *E. coli*, *E. aerogenes*, *K. pneumoniae* and *E. cloacae* represented 56, 21, 9 and 8% of the ESBLE, respectively. 48% of all ESBLE were considered as community-acquired (CA) (73% of *E. coli* isolates). Majority of ESBL types were CTX-Ms (53%) (with predominance of CTX-M-15 [77%]), TEM (31%; TEM-24, TEM-52) and SHV (12%; SHV-4, SHV-12). Among ESBLE, co-resistance to ciprofloxacin, cotrimoxazole and aminoglycosides was 79, 70 and 51% respectively. CMY and DHA-like ampC were detected in *E. coli* (6%) and in *K. pneumoniae* (10%). No carbapenemase was detected in this survey. Spread of CTX-M-15 *E. coli*, *K. pneumoniae* and SHV-12/CTX-M-9 co-producing *E. cloacae* occurred in several hospitals.

Conclusions: In comparison to a previous study carried out in 2006, we observed a marked increase in CA CTX-Ms producing *E. coli* and a parallel decrease of the TEM-24 *E. aerogenes* Belgian epidemic clone. The emergence of isolates simultaneously containing ESBLs, AmpC and resistance mechanisms to fluoroquinolones and aminoglycosides is of concern and highlights the need for further surveillance at national level in Belgium.

P975 Clonal dissemination of CTX-M-15 producing *E. coli* in Belgium: a multi-centre surveillance study

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Objectives: CTX-M ESBL-producing strains of *E. coli* are expanding world wide. As part of a national surveillance project, we analysed the diversity of extended spectrum-B-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 (cefotaxime and ceftazidime + clavulanic acid). ESBL 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 group in 44 isolates (including 33 CTX-M-15, 10 CTX-M-1 and 1 CTX-M-3) 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 phylogroups 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. ISEcp1 and IS26 was found upstream to CTX-M-15 in 29 and 4 isolates respectively and uptstream to CTX-M-1 in 6 and 4 isolates, respectively. None of these IS could be found upstream to CTX-M-3. Orf513 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.

P976 Predominance of virulent CTX-M producing strains in extended spectrum B-lactamase producing *E. coli* in a Belgian university hospital

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Objectives: The spread of CTX-M producing *E. coli* (CTX-M-EC) is of public health concern. We analysed the molecular epidemiology and the

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%), and 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, cotrimoxazole 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.

P977 Polyclonal spread of extended-spectrum β-lactamase producing *Enterobacter cloacae* at a Belgian university hospital

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Objectives: Nosocomial outbreaks caused by extended-spectrum β-lactamase-producing *Enterobacter cloacae* (ESBL-EC) have recently been reported in several countries worldwide. We describe here the clinical, epidemiological and microbiological characteristics of ESBL-EC isolates recovered at a large Belgian university hospital.
Methods: We performed a prospective surveillance of all hospitalised patients with infection or colonisation by an ESBL-EC strain. Production of ESBL was determined by decreased susceptibility or resistance to expanded-spectrum cephalosporins (Ceph-R) with the BD Phoenix System and by combination disc method using Rosco tablets. ESBL types were further characterised by multiplex PCR targeting the TEM, SHV, CTX-M coding genes families and amplicons were sequenced. All isolates were identified up to the subspecies level within the *E. cloacae* complex and the clonal relatedness of the strains was determined by PFGE using Xba1 restriction enzyme.
Results: From Oct. 2006 to Sept. 2007, 98 of 396 *E. cloacae* isolates were identified as Ceph-R. 25 of these strains (26%) were ESBL-positive; 6 isolates expressing SHV-5/-12 and 16 other harbouring CTX-M (13 CTX-M-9 and 3 CTX-M-2) alone (6) or in association with SHV-5/-12 (10). Most ESBL-EC displayed associated resistance to quinolones and to aminoglycosides. The ESBL positive isolates were identified into four subspecies including 12 *Enterobacter hormaechei* subsp. *oharae*, 6 *E. hormaechei* subsp. *steigerwaltii*, 2 *E. hormaechei* subsp. *hormaechei* and 7 *E. cloacae* sensu stricto. PFGE disclosed at least 20 different patterns. Medical records of the 25 patients revealed 15 admissions to an intensive care unit (ICU) prior to the isolation of the strains, 11 infections and 6 deaths (including 4 attributable).
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*.

P978 Epidemiology of ESBL producing *Klebsiella pneumoniae* isolates from a paediatric hospital

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Objective: The extended-spectrum β-lactamases (ESBLs) are the main mechanism of resistance to oxyimino-β-lactams among Gram-negative clinical isolates, especially in *K. pneumoniae*, an organism responsible for community- and hospital acquired infections. During 2004–2007, in the Childrens Memorial Health Institute (CMHI) the frequency of *K. pneumoniae* among Gram-negative isolates from blood varied between 15–28%, but the frequency of ESBLs reached 37% in 2006 and decreased to 16% in 2007. The aim of this study was the epidemiologic analysis of *K. pneumoniae* isolates producing ESBLs obtained from hospitalised children in the period 2004–2007.
Methods: 62 clinical isolates of *K. pneumoniae* producing ESBL were obtained from CMHI. MICs of selected β-lactams were determined by the agar dilution method agar as recommended by CLSI. PCR analysis was performed using primers specific for blaCTX-M, blaSHV and blaTEM. Sequencing was carried out using ABI PRISM 310 DNA sequencer and analyzed using CLC Free Workbench 4.0 and Finch TV Program. Genotyping was done using PFGE.
Results:. All 62 *K. pneumoniae* ESBL+ isolates gave a positive result for blaCTX-M genes. Among them 51 had the identical sequence typical for CTX-M-3 enzyme and eleven had CTX-M-15 enzyme. The MIC90 values for ceftazidime and ceftazidime plus clavulanate were equal to 64 mg/L and 4 mg/L, respectively. The MIC90 values for cefotaxime and cefotaxime plus clavulanate was equal to 128 mg/L and 4 mg/L respectively. Majority of *K. pneumoniae* strains produced additionally TEM enzymes (37) and/or SHV enzymes (20). TEM-47, TEM-48, TEM-86, TEM-130, TEM-132, SHV-1 and SHV-5 enzymes were detected. PFGE analysis showed that only 37% of *K. pneumoniae* strains were clonal. The presence of five clones with 100% identity and two clones with 90% and 87% identity, respectively, were found.
Conclusion: Epidemiological analysis of *K. pneumoniae* ESBL producing clinical isolates showed that they produced CTX-M enzymes and majority of them additionally TEM and/or SHV. Only 37% of them showed clonal relationship.

P979 Emergence of ESBL-producing *Escherichia coli* and *Klebsiella* spieces isolates in Lithuania

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Objectives: the main purpose of the study was to investigate the frequency of ESBL-producing *E. coli* and *Klebsiella* clinical isolates in Lithuania and to determine the types of ESBL encoded.
Methods: the antibiotic susceptibility of 221 nonduplicate clinical isolates, including 124 *E. coli*, 86 *K. pneumoniae* and 11 *K. oxytoca* collected in three regional hospitals during the period 2007–2008 was determined by using Sensititre plates (GN1F). The isolates with reduced susceptibility to oxyimino-cephalosporins were examined for ESBL production using Confirmatory MIC plates (ESB1F, Sensititre). ESBL producers were analyzed by PCR to detect the ctx-M, shv, oxa genes. ESBL type was determined by nucleotide sequencing (CTX-M) and NheI restriction typing (SHV-ESBL).

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 GEN TOB NIT (N = 16) and for *E. coli* CTX-M-15 producers GAT CIP TOB (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 GEN TOB AMI NIT SXT.
Conclusion: the first survey revealed the high frequency of ESBL producing *E. coli* and *Klebsiella* clinical isolates and a high predominance of SHV-ESBL, CTX-M-15, CTX-M-2a in *K. pneumoniae* and CTX-M-15, CTX-M-2a in *E. coli* isolates in Lithuania.

P980 Dissemination of ST274 *Klebsiella pneumoniae* epidemic clone adapted to the newborn and adult hospital settings by producing SHV-2a or CTX-M-15 type extended spectrum β-lactamases

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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 profile 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, plazmid 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 ST274.
Conclusion: 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.

P981 Identification of PER-1, CTX-M-3, CTX-M-15, SHV-2, TEM-3 and TEM-15 extended-spectrum β-lactamases in chromosomal AmpC-producing Enterobacteriaceae isolates from cancer patients in Bulgaria

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Objective: To characterise extended-spectrum β-lactamase (ESBL) genes present in chromosomal AmpC-producing enterobacterial isolates recovered in a Bulgarian cancer hospital.
Methods: Screening for genes encoding ESBLs (blaPER-1, blaTEM, blaSHV, blaCTX-M, blaVEB-1 and blaGES-1) 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 morganii* 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.

P982 Prevalence of CTX-M producing *Klebsiella pneumoniae* in hospitals versus community

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Objectives: This study was conducted to detect and analyze the presence of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* within clinical isolates of both community- and hospital-based patients in Croatia and to characterise bla genes in these isolates.
Methods: 648 consecutive non-duplicate *K. pneumoniae* isolates from 26 laboratories in 21 Croatian cities were collected from October 2006 to January 2007. 128 *K. pneumoniae* isolates were selected for further study on the basis of positive double disc diffusion test for ESBL production. After determination of minimal inihibitory concentration (MIC), transfer of bla genes was performed by conjugation assay with *E. coli* J62–2 (RifR) (NCTC 50170) as the recipient. PCR was used to detect alleles encoding CTX-M enzymes. 33 producers were compared by analysis of banding patterns generated by pusled-field gel electrophoresis (PFGE) of Xba I-digested genomic DNA.
Results: Percentage of ESBL-positive isolates from hospitals was 56, and 44% originated from community. CTX-M was detected in 33 (25.8%) isolates. 16 hospital acquired isolates were CTX-M positive (22.2%) and 17 CTX-M positive community isolates were detected (30.4%). Pulsed-field gel electrophoresis analysis demonstrated three major similarity groups according to Tenover criteria, each in different geographical region.
Conclusions: According to our results (25.8%) of ESBL isolates were CTX-M positive. PFGE analysis revealed three major similarity groups which have shown specific geographical distribution. CTX-M positive *K. pneumoniae* strains will be further characterised using multiplex PCR and sequencing.

P983 Antimicrobial susceptibility testing of extended-spectrum β-lactamase producing Enterobacteriaceae isolated from Macau, China

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Objective: ESBLs emerged in the 1980 s 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 ceftazidime or cefotaxime in combination with clavulanate 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 discrimates between CTX-M-gp 1, CTX-M-gp 2, CTX-M-gp 8, 25/26, and CTX-M-gp 9 of 260, 341, 207 and 293 bp respectively.
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.

P984 Molecular characterisation and epidemiology of Enterobacteriaceae isolates other than *Escherichia coli* and *Klebsiella* spp. that are non-susceptible to extended-spectrum cephalosporins in Thailand

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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, ceftazidime, 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.

P985 Rampant resistance to β-lactam antibiotics in previously susceptible Enterobacteriaceae: the Kwazulu-Natal experience

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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 multicentre surveillance study instituted in 16 hospitals at 3 progressive levels of health care (district, regional, and tertiary) where microbiology laboratories collected 100 consecutive, non-repetitive 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, CTX-M-37, 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

P986 Medical intervention at immigrant camps in Greece

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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 data base 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 (26.2%) 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.

P987 Protocol for congenital Chagas disease control in Florence, Italy

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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 a nearly 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.

P988 Travel-associated enteric fever: a review of demographics, clinical features, laboratory findings, treatment and outcomes in a UK district general hospital

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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% rigors), gastrointestinal (90% with 68% diarrhoea, 63% nausea/vomiting, 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 44% 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.

P989 Effective tick removal with a fishing line knot

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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.

A 2 mm tick struggling to free from fishing wire node after it has been removed from the thigh of an infant.

P990 Phylogenomic and subproteomic approaches to gain new insights into the mitochondrion features of the *Cryptosporidium parvum* parasite

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Objectives: *Cryptosporidium* spp. is a protozoan parasite that causes widespread diarrhoeal 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.

P991 Alpha-tocopherol transfer protein gene disruption confers resistance to protozoan infections

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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 parasitaemia 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 parasitaemia 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.

P992 Iron supplementation for children in malaria-endemic areas: systematic review and meta-analysis

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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 hypoendemic to holoendemic malaria regions. Trials comparing orally administered iron+/-folic-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 gr/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

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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 (LBRF) 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 spriochaetes 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 mutS, 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 LBRF patient clinical material. The apparent phylogenetic overlap between LBRF and TBRF spirochaetes was also confirmed.

P994 *Entamoeba histolytica* or dispar through PCR and Elisa II?

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Objectives: Amebic 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. dispar*) 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. dispar* 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. Adhesin was examined in our study group through Teclab E. Histolytica II ELISA kit. Parasite DNA was investigated through multiplex PCR method, using primers specific to *E. histolytica* (EhP1 and EhP2) and E. Dispar (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. dispar*) 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)

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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 precondition 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.

P996 Cytoadherence and genotype of *Plasmodium falciparum* strains from symptomatic children in Franceville, south-eastern Gabon

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Background: *Plasmodium falciparum* causes severe clinical manifestations by sequestering parasitised red blood cells (PRBC) in the microvasculature of major organs such as the brain. This sequestration results from PRBC adherence to vascular endothelial cells via erythrocyte membrane protein 1, a variant parasite surface antigen.
Objective: To determine whether *P. falciparum* multiple genotype infection (MGI) is associated with stronger PRBC cytoadherence and greater clinical severity.
Methods: Nested PCR was used to genotype *P. falciparum* isolates from symptomatic children and to distinguish between single genotype infection (SGI) and MGI. PRBC cytoadhesion was studied with cultured human lung endothelial cells (HLEC).
Results: Analysis of two highly polymorphic regions of the merozoite surface antigen (MSP)1 and (MSP)2 genes and a dimorphic region of the erythrocyte binding antigen (EBA-175) gene showed that respectively 21.4% and 78.6% of the 42 children had SGI and MGI. It also showed that 37 (89%) of the 42 PRBC samples expressed MSP1 allelic family K1. Cytoadherence values ranged from 58 to 1811 PRBC/mm2 of HLEC monolayer in SGI and from 5 to 5744 PRBC/mm2 in MGI. MGI was not associated with higher cytoadherence values or with more severe malaria.
Conclusions: These results suggested that infection of the same individual by multiple clones of *P. falciparum* does not significantly influence PRBC cytoadherence or disease severity and confirmed the predominance of the MSP1 K1.

P997 Analysis of pathogenicity of *Plasmodium falciparum* field isolates using a coculture model of human endothelial cells/*P. falciparum*

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Plasmodium falciparum infection can lead to a life threatening disease. However, the pathogenetic mechanisms of severe manifestations are not fully understood. Here we investigated whether *P. falciparum*-parasitised red blood cells (PRBC) with clinical malaria can induce endothelial cell (EC) apoptosis in vitro. In all 45 subjects tested, PRBC that cytoadhered to HLEC could be found albeit to a variable degree. In contrast, PRBC that induce HLEC apoptosis was found only in 9 subjects. Interestingly, apoptosis was significantly associated to the presence of neurological signs ($P = 0.02$) but there was no significant association between apoptosis and the severe malaria clinical status as a whole (uncomplicated versus severe malaria; $P = 0.11$). The intensity of apoptosis was variable and was linked to parasite load (Spearman rho=0.41, $P = 0.005$). The degree of cytoadherence was higher among individuals with apoptosis (median, 25%-75% interquartile interval: 937, 610–1811 PRBC/mm2HLEC) than individuals without apoptosis (median, 25%-75% interquartile interval: 437, 130–830.5 PRBC/mm2HLEC). This difference was significant (Mann-Whithney U test, $P = 0.017$), however, the correlation between the degree of cytoadherence and the capacity to induce apoptosis was not significant (rho= 0.14, $P = 0.37$) suggesting a qualitative rather than a quantitative relationship.
PRBC single (SGI) and multiple genotype infections (MGI) were determined using nested polymerase chain reaction targeting two highly polymorphic regions of the merozoite surface antigen 1 and 2 and a dimorphic region of the erythrocyte binding antigen 175 loci. The results demonstrated that infection of the same individual by multiple clones of *P. falciparum* does not significantly influence PRBC cytoadherence or apoptosis induction in HLEC. However, MGI is inversely correlated with in vivo synchronisation.
Analysis of the whole transcriptome from apoptogenic versus non apoptogenic *P. falciparum* revealed 59 genes putatively associated with the induction of EC apoptosis. Finally, silencing of parasite gene expression with specific double-stranded RNA was performed on 8 selected genes; 5 of these, termed "*Plasmodium* apoptosis-linked pathogenicity factors" (PALPFs), were found to be linked to parasite apoptogenicity. Strategies of using these new pathogenicity factors as anti malaria drugs or vaccine targets will be discussed.

P998 Coagulation abnormalities in imported malaria-clinical and laboratory findings

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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-paetechial 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.

P999 Awareness, possession and use of insecticide-treated net for prevention of malaria in children under five in Abeokuta, Nigeria

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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.

P1000 **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**

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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-histaminic products to relieve the itching.

P1001 **Entomological survey of plebotomine sand flies in a focus of visceral leishmaniasis in a regional centre of Portugal (Coimbra municipality)**

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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 Disese 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. sergenti*) 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. sergenti* (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.

P1002 ***Brucella melitensis*** **endocarditis accompanied with** ***Candida albicans*** **in a patient with prosthetic valve**

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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 37.5ºC, hepatosplenomegaly and in cardiac examination a grade 3/6 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.

P1003 ***Trypanosoma cruzi*** **infection diagnosis in a Spanish health department during 2008**

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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.

Methods: A descriptive study was conducted through all 2008. 645 sera from 627 patients from the 7th Health Departament in Valencia were sent to our tertiary referral hospital Microbiology laboratory (La Fe University Hospital) for Chagas' disease screening. The samples were tested for anti-*Trypanosoma cruzi* antibodies (IgG) using 2 different enzyme-linked immunosorbent assays (ELISA). Positive sera were then confirmed with an immunofluorescent assay (IFA) and a particle gel immuno assay.
Results: Out of 627 patients, 9 were Spanish citizens who had previously been in Southamerica; their median age was 38 (range 25–67) years old and 55.5% were women.
618 patients had Latin American origin; grouped by age, 85 (13.8%) were less than 15 years old, 71 (83.5%) being newborns in Spain from Latin American mother. 533 patients were older than 14 (range 15–64; median age 28 years old) of whom 93.1% were women. Most patients were pregnant women (80.7%). The most frequent countries of origin were Bolivia (35.5%), Ecuador (32.1%) and Colombia (14.1%).
All non-neonatal patients with positive *T. cruzi* serology (n = 49; 8.8%) were from Latin America (68.2% from Bolivia); 28 were pregnant women (57.1%).
Out of all children born to Latin American women, 23.3% (n = 17) had positive *T. cruzi* serology at birth which corresponded to 6.5% (n = 28) of the total of pregnant women studied.
Conclusions: Prevalence of Chagas' infection is high in pregnant women from Bolivia. Screening should be carried out in pregnant women from Latin America in our country for follow-up and early treatment of infected children. The introduction of screening programmes is important to determine the relevance of the disease in fertile women and to interrupt vertical transmission, but adult men should not be forgotten.

P1004 Visceral leishmaniasis in Serbia – treatment problems

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Visceral leismaniasis 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 regions of Serbia.
Methods: During 2001–2008 periods in our department we treated 25 patients safer from visceral leismaniasis. 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 mails 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. Spelnohepatomegaly 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 amphothericin B. All of them were treated during 15–28 days, given intravenously for a total dose of 20 mg/kg. After two courses of amphothericin 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 amphothericin 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 amphothericin B.

P1006 Cystic echinococcosis: decreasing prevalence in Central Greece

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Objectives: Cystic echinococcosis (CE) remains an endemic parasitosis worldwide, with Greece traditionally considered as one of the endemic European countries. We sought to evaluate prevalence trends of CE in a hyper-endemic for zoonoses area of Central Greece, as detected through abdominal ultrasound.
Methods: Abdominal ultrasound records of patients tested at the Radiology Department of the General Hospital of Trikala, Thessaly, in Central Greece, for the period 2001–2008 were evaluated. The presence of simple hepatic cysts and liver haemangiomas was studied in parallel.
Results: Of the 47045 total ultrasound tests performed in the period 2001–2008, a total of 189 tests revealed abdominal hydatid disease, representing 153 individuals with cystic echinococcosis, indicating prevalence below 4 per 1000. Prevalence of haemangiomas reached 5 per 1000, and that of simple hepatic cysts 13 per 1000. Dividing the study period in two sub-periods (2001–2004 and 2005–2008), a statistically significant trend towards decreasing annual incidence was noted for abdominal CE.
Conclusion: The prefecture of Thessaly in Central Greece is hyper-endemic for numerous zoonoses, CE included, due to its population characteristics (rural orientation, agricultural basis of the economy). Despite a generally high prevalence, a statistically significant decreasing trend in annual incidence was noted during the second half of the study period. In conclusion, the implementation of a national campaign for control of cystic echinococcal disease in both animals and human (started in Greece in 1984), has led to decreasing annual detection rates of CE in Central Greece.

P1007 The prognostic and diagnostic value of Western Blot test in cystic echinococcosis

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Aim: Cystic echinococcosis (CE) is a zoonosis and surgery is the treatment of choise and in certain cases supply complete cure. Surgery have problems like the inadequate cyst removal and relapses from ruptured and 'new' cysts not previously detected. The pre and post-surgical follow-up (PS) of hydatidosis performed by indirect haemagglutination (IHA), ELISA and Western Blot IgG (WB) tests. The antibodies can be detected for longer years and the usefullness of ELISA and IHA methods are limited for PS. We aimed to show the antigenic patterns of operated (OP) and non-operated patients with hydatid cysts (NOP) and also evaluate the importance of the antigenic differences for the PS of the patients. We also aimed to detect the specificity of *Echinococcus granulosus* WB kit.
Method: A total of 80 patients, 25 NOP, 33 OP and 22 healty controls (HC) with no known chronic disease were included. ELISA method was used. Cystic hydatid IgG antibodies were detected positive in serum samples of the all patients and no positivity for IgG antibodies were detected in serum samples of the HC. The antibodies against to the non specific p39, p24/26 kDa (genus specific but cross reactive with other species), p16/18 and p7 antigens specific for *E. granulosus* were investigated with WB (Euroimmun Labordiagnostica, Germany) kit. Serum samples with antibodies against to the p39 were considered negative and serum samples with antibodies against to the p7 were

considered positive. Serum samples which have a weak bands for p16/18 ve p24/26 were considered negative, on the other hand the serum samples with strong bands for the same antigens were considered as suspicious and need to follow up.
Results: Antibodies against to the p7 antigen were detected in 24 (96%) of NOP and 18 (54.5%) of OP. No antibody was detected against to the p7, p16/18 and p24/26 antigens. It was detected a statistically higher significant difference between OP and NOP for p7 antigen ($p < 0.01$). The calculation of sensitivity and specificity of diagnostic Western Blot IgG kit were performed with serum samples of 25 NOP and 22 HC. The sensitivity and specificty of the WB test kit were calculated as 96% and 100%, respectively.
Conclusion: As a result of we suggest that antibodies against p7 antigens can be used for the diagnosis and PS of the patients with hydatid cysts because of the presence of antibodies against p7 antigens in serum samples of NOP and the decrease of antibodies against p7 antigens in the serum samples of OP.

P1008 Evolutive pattern of trichinellosis in patients of a Roma community

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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 rromi 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 Iecea 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 38ºC, 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 members of gipsy communities that consume pork in poor hygenic conditions. The study of evolutive and clinical pattern of trichinellosis in a population at risk increases the awareness regarding the implementation of efficient prophylaxis and proper treatment.

P1009 A Romanian winter outbreak of trichinellosis due to consumption of pork contaminated with *Trichinella spiralis*

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Objectives: Extensive characterisation of a trichinellosis epidemic in a Romanian endemic region due to consumption of contaminated pork.
Methods: In January 2008, an outbreak of trichinellosis due to consumption of infected pork involved 15 people in localities Carpinis 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 occured 17 days following the consumption of the infected meat and all patients presented headache, eyelid and lower limb oedema, myalgia, fever, diarhhoea 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.

P1010 Expression of K2S form of human tissue plasminogen activator in trypanosomatid protozoan *Leishmania tarentolae*

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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 pFXmlsap1.4hyg-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 (pFXmlsap1.4hyg-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 SSurRNA (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 heterologus 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

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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 5×10^6 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

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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

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Objective: In order to study relationships between an established bacterial community and its specific bacteriophages, we developped an animal model using an enteroaggregative *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 enteroaggregative *E. coli* nº55989 strain (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 55989 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* 55898 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

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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 induction; lungs were taken for histological analysis and bacterial count.
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 ($4\times10^6 \pm 2.5$ CFU/lung vs $3\times10^4 \pm 2$ CFU/lung). In contrast, 30% of mice subjected to moderate exercise resulted infected only. The bacterial size was $2\times10^4 \pm 1.4$ 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.

P1015 Anti-inflammatory effect of adjuvant glycerol in experimental pneumococcal meningitis

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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 (carboxymethylcellulosis) 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 edema. Furthermore, concentrations of matrixmetalloproteinase (MMP)-2 and MMP-9 as well as activity of myeloperoxidase (MPO) were assessed in the cerebrospinal fluid (CSF) obtained at 24 h and 40 h after infection. In adult mice, hearing thresholds were determined using auditory brainstem potentials. Then, animals were sacrificed. Mouse temporal bones were dissected and evaluated for histomorphology.
Results: In the infant rat model, glycerol reduced MPO-activity at 24 h after infection and lowered the concentration of MMP-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. neutrophils) invaded the CSF. MMP-2 and particularly MMP-9 were shown to contribute to the development of brain injury in bacterial meningitis. Thus, the decrease of MMP-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.

P1016 Efficacy of garenoxacin in a skin infection model caused by methicillin-resistant *Staphylococcus aureus* in diabetic mice

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Objectives: Garenoxacin (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 Iar-+Leprdb/+Leprdb mice were used as type II diabetic mice. After shaving the hair on an area of the thigh under anaesthesia, mice were infected by placing a 5 ul droplet containing ca. 10^5 CFU of MRSA F-3410 (MIC: GRN 0.0313 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 $\pm$ 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 ug Eh/g ($N = 3$, 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.

P1017 Beneficial effects of erythropoietin in experimental pneumococcal meningitis

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Objectives: Up to half of patients surviving pneumococcal meningitis suffer from neurologic sequelae including hearing loss and learning difficulties. In experimental pneumococcal meningitis, these functional deficits are associated with loss of neurons in the cochlea and the dente gyrus of the hippocampus for hearing loss and learning deficits, respectively. Erythropoietin (EPO) is neuroprotective in other paradigms of brain injury. Here, the effects of EPO on hearing loss and hippocampal neurogenesis were evaluated in an infant rat model of pneumococcal meningitis.
Material and Methods: Pneumococcal meningitis was induced in 11 day old Wistar rats by intracisternal injection of *Streptococcus pneumoniae*. Control littermates were injected with sterile saline. Eighteen hours after infection, antibiotic therapy (ceftriaxone, 100 mg/kg s.c. bid) was initiated. Animals were randomised for treatment with EPO (500 U/30 g, i.p) or an identical volume (150 microL) of saline daily for 5 consecutive days after infection. In order to quantitatively assess neurogenesis by measuring cell proliferation, bromodeoxyuridine (BrdU, 50 mg/kg, i.p) was administrated for 3 consecutive days, directly after the infection or 3 weeks later. Hearing capacity was tested in both ears by assessing auditory brainstem response 3 weeks after infection and animals were sacrificed subsequently for histomorphometry of the brain

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 (p = 0.04, t-test) increased neurogenesis. The rate of cell proliferation in the dentate gyrus 3 weeks after infection was 262±123 BrdU positive cells/mm^2, (n = 5) in infected animals treated with EPO vs 148±21 cells/mm^2 (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

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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

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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 heart, 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 In vitro profiling of pramiconazole against a panel of moulds and yeasts and in vivo activity against *Microsporum canis* and *Candida albicans*

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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 itraconazole 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 spectro-photometric 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

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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 30 g 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 statical 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, 39.1and 42.4 respectively. Comparison of lipid profile in treated and control group showed cholesterol, triglyceride and LDL had reduced in treated group significantly ($p = 0.029$, $p < 0.001$, $p = 0.015$ 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

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Objectives: Vigorous granulomatous and fibrosing reactions to parasite eggs specify the pathological picture of Schistosoma mansoni infection as one the leading causes of liver fibrosis and portal hypertension. Current antihelmintic 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 NMRI mice 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 (sirius 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, col1a2) 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, $p < 0.05$). 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

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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 (35 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 16 h incubation time and development of meningitis, rabbits were seperated into three groups. Group 1 was given vancomycin 20 mg/kg every 12 h, Group 2 was given linezolid 20 mg kg every 12 h, and Group 3 was control. Cerebrospinal fluid bacterial counts were measured at 16 and 40 h. 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 37ºC for 24 h. 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 χ^2 test. A p-value less than 0.05 was considered significant. The study was approved by the local ethical commitee 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 16 h. Bacterial counts were similar in all groups at 16 h. At 40 h 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 40 h 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.

Group	Bacterial count ($\log_{10}$ CFU/mL)	
	16 h	40 h
Vancomycin	4.33±1.42	3.09±2.05
Linezolid	3.43±0.91	2.22±2.08
Control	3.85±0.26	5.14±0.99

P1024 Dalbavancin and rifampin against methicillin-resistant *Staphylococcus aureus* in an experimental foreign-body infection

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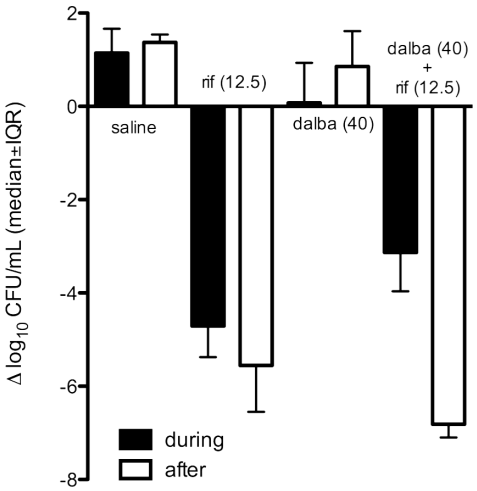
Objectives: Implant-associated infection is a devastating complication caused by biofilms adhering to surface of devices. We investigated the in-vitro susceptibility, pharmacokinetics and treatment efficacy of dalbavancin (DAL), alone and in combination with rifampin (RIF) in a foreign-body MRSA infection model.

Methods: MRSA (ATCC 43300) MIC and MBC was determined by macrodilution method. Time-kill curves were determined in the logarithmic and stationary growth-phase during 48 h. Tween 80 (final concentration 0.002%) was added to all solutions in glass tubes. A

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 h × 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 ug/ml and MBC was >20 ug/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 ug/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.



P1025 Antibacterial activity of NXL103 (linopristine-flopristine) against intracellular *Staphylococcus aureus* and efficacy following per oral administration in murine models of systemic infection

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Objectives: NXL103 (linopristine-flopristine) 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 J774 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 7×10^5 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 3×10^7 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 clarithromyin 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.

P1026 Efficacy of vancomycin, linezolid, and cotrimoxazole in the treatment of an experimental pneumonia model caused by methicillin-resistant *Staphylococcus aureus*

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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]; t>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 uninfected 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 (t>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/MIC0–24 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 therapeutical alternative in the pneumonia caused by MRSA.

Group	Doses (mg/kg)	No.	Log cfu/g lung (mean±SD)	Negative blood culture (%)	Survival (%)
MRSA30					
CON	–	14	8.93±0.78	3 (21.4)	12 (85.7)
VAN	30	14	6.67±3.01	9 (64.3)[a]	13 (92.9)
	110	13	3.25±1.59[a,b]	13 (100)[a,b]	10 (76.9)
LZD	30	16	2.87±1.86[a,b]	15 (93.8)[a]	16 (100)
COT	10/50	15	3.18±2.04[a,b]	15 (100)[a,b]	15 (100)
MRSA33					
CON	–	15	8.62±0.72	6 (40)	10 (66.7)
VAN	30	15	5.76±2.43[a]	10 (66.7)	15 (100)[a]
	110	16	3.97±1.52	16 (100)[a,b]	12 (75)
LZD	30	15	1.59±1.40[a,b,c,d]	14 (93.3)[a]	15 (100)[a]
COT	10/50	15	3.08±2.49[a]	13 (86.7)[a]	15 (100)[a]

[a] $p < 0.05$ 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.

P1027 Attenuation of bacteraemia by treatment with serotype-specific antibodies diminishes hippocampal neural apoptosis during experimental pneumococcal meningitis

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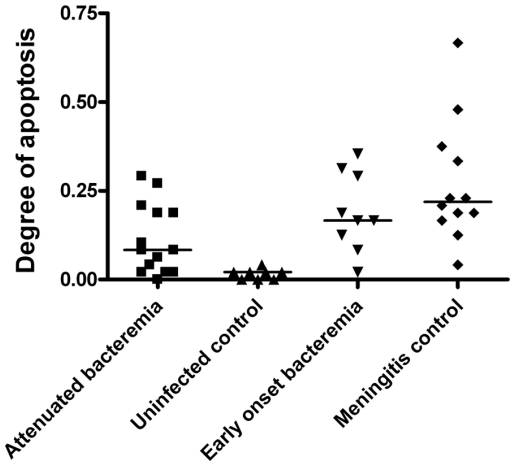
Objectives: Bacteraemia and systemic complications contribute to the development of brain damage and may be directly responsible for up to half of all fatal cases of pneumococcal meningitis.
Methods: Using an adult rat pneumococcal meningitis model, the impact of accompanying bacteraemia on the development of hippocampal injury was studied in the following groups: Meningitis controls (n = 11), meningitis with early onset bacteraemia from concomitant iv infusion of pneumococci (n = 9), meningitis with attenuated bacteraemia 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 bacteraemia by antibody treatment resulted in significantly reduced apoptosis (0.08 (0.02–0.20), P = 0.01).
Conclusion: Our results suggests that accompanying bacteraemia has a significant role in development of hippocampal injury during pneumococcal meningitis.



P1028 Efficacy of oritavancin in the rat haematogenous pneumonia model

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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 1.2×10^7 colony-forming units (CFU)/mL of MRSA strain NRS123 (USA 400) (ORI MIC = 0.06 mg/L) enmeshed in 0.08% agar beads (as described by Sawaï 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 the MRSA concentrates and remains in the lungs. ORI regimen showed efficacy 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 ($p \leqslant 0.036$ 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

P1029 Investigation of carbapenem heteroresistance among susceptible *Acinetobacter baumannii*

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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.

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. Imipenem-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.

P1030 In vitro activities of various antimicrobials alone and in combination with imipenem against carbapenem-resistant *Acinetobacter baumannii* blood isolates

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The prevalence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) related infections continue to increase, however, therapeutic options for the 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 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 impenem, 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.

P1031 Multidrug-resistant *Acinetobacter*: is colistin still effective?

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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 Adult Hospital, 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 (IMP), 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-sulfomethoxazole (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. lwoffii*. Forty five (36.3%) of isolates were from patients in ICUs. MIC50 and MIC90 (μg/ml) values of isolates were found 32 and 128 (IMP), 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. Sixty four (51.6%) were positive for IMP-EDTA combined disk test. Overall, 51 (%41) *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 were 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.

Conclusions: 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* isolates, the study suggests that colistin may still be an alternative therapeutic option in MDR *Acinetobacter* isolates.

P1032 The role of the MDR efflux pumps of Gram-negative rods in removing "non-antibiotic" drugs

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A potential role of non-antibiotics in the treatment of multidrug-resistant Gram-negative infections has been investigated recently. In the case of some medicines like antipsychotic or cardiovascular drugs, a direct antibacterial activity was shown. Moreover, phenothiazines inhibit efflux pumps in Gram-positive bacteria. On the other hand, salicylate is the natural substrate for the efflux pumps in some Gram-negative rods eg. Burkholderia cenocepacia and can induce efflux-mediated resistance.

In our study, the activity of 10 non-antibiotic drugs and their 6 active substances was evaluated against non-fermentative Gram-negative rods and Enterobacteriaceae (12 standard and 59 clinical isolates) in the presence and absence of pump inhibitors (Phe-Arg-b-naphthylamide or reserpine). Influence of analyzed drugs on activity of efflux pumps in Gram-negative rods was studied by changing the strain susceptibility to nalidixic acid in the presence of tested medicines and +/– pump inhibitor.

Results: The highest activity of alendronate and drugs possessing this active substance (Osalen®, Rekostin®) was shown (MIC 100–800 mg/L) for majority of strains. Only *B. cepacia* and *Acinetobacter baumannii* were resistant. Also drug Amitryptylinum® (containing amitryptyline) was very active (MIC 200–800 mg/L) against rods, except *B. cepacia* and *P. aeruginosa*. Moreover, the decrease of MIC values (4–64-fold) of some active substances (drugs) like alendronate (Osalen®, Rekostin®), ticlopidine (Ticlo®, Apo-Clodin®) and drugs as Amitryptylinum® and Niglostin® in the presence of Phe-Arg-b-naphthylamide (but not reserpine) was obtained for *E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Stenotrophomonas maltophilia* and *A. baumannii*. Generally, in the presence of drugs as Osalen®, Apo-Clodin®, Amitryptylinum® and Niglostin® the change of strains sensitivity to nalidixic acid was not observed. Only for 3 *A. baumannii* strains and one *E. coli* the decrease of nalidixic acid MIC in the presence

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, amitriptyline 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.

P1033 Comparative susceptibility of European Gram-negative pathogens to ceftobiprole, ceftazidime and cefepime

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Objectives: Ceftobiprole (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 Ceftobiprole Local Antibiotic Susceptibility Surveillance Study (CLASS) compared the susceptibility of common pathogens causing serious infections in hospitalised patients. Here we report the comparative activity of BPR, ceftazidime (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, blood stream 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 4 mg/L but BPR had the lowest MIC50 of 0.06 mg/L compared with 0.125 mg/L for CFP and 0.25 mg/L for CAZ. Against *E. coli*, *H. influenzae* and *Klebsiella* spp., BPR had a lower MIC90 than CAZ and CFP (0.125, 0.5 and 0.25 mg/L vs. *E. coli*; 0.125, 0.25 and 0.25 mg/L vs. *H. influenzae*; 0.125, 1 and 0.25 mg/L vs. *Klebsiella* spp. respectively). Against *Enterobacter* spp., BPR had a lower MIC90 (4 mg/L) than CAZ (128 mg/L) but two-fold higher than CFP (2 mg/L). Against *P. aeruginosa*, BPR, CAZ and CFP MIC90 were 16 mg/L, 8 mg/L and 8 mg/L respectively.
Conclusion: BPR showed activity comparable to CFP but generally more active than CAZ against a range of GNPs.

P1034 Comparative susceptibility of European Gram-negative rods to doripenem, imipenem and meropenem

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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 break points for DPM.
Results: The MIC90 against all isolates was 2, 4 and 32 mg/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 0.125 mg/L for DPM & MPM but 0.5 mg/L for IPM. DPM had the lowest MIC90 against Psuedomonas spp. at 8 mg/L with MPM at 16 mg/L and IPM at $\geqslant$ 64 mg/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.

P1035 Temocillin in vitro activity against ESBL- and AmpC producing Gram-negative bacteria isolated in tertiary-care hospital

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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 cefepime, 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 30 ug temocillin (Neosensitabs, Rosco) was used and inhibition zones were recorded.
Results: There were 22 *Enterobacter cloacae* (16 AmpC), 12 ESBL *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 occured 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.

P1036 Trends in susceptibility of Gram-negative pathogens isolated from intra-abdominal infections in North America from 2003 to 2007 – the SMART Study

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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 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 (%S) 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.

P1037 Trends in susceptibility of Gram-negative pathogens isolated from intra-abdominal infections in Europe from 2003 to 2007 – the SMART Study

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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 n's of *E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, *E. aerogenes*, *C. freundii*, *M. morganii*, *P. mirabilis*, and *P. aeruginosa* were 1011/1291, 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.

P1038 In vitro susceptibility of quinolone-resistant *Escherichia coli* isolates with and without producing extended-spectrum β-lactamase to fosfomycin trometamol

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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.

P1039 In vitro activity of fosfomycin against multidrug-resistant Enterobacteriaceae isolates from a Belgian hospital

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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 *Escherichia coli* and *Klebsiella* spp..
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, Biomérieux, 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 $\leqslant$ 64 μg/ml, resistant $\geqslant$ 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 with MIC50 of 3 μg/ml and MIC90 of 16 μg/ml. Subsequently, the ESBL-producing *Klebsiella* spp. strains had a MIC range of 1.5 to 96 μg/ml, with MIC50 of 16 μg/ml and MIC90 of 48 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 MIC50 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-produding *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.

P1040 In vitro activity of ertapenem in comparison with imipenem against Enterobacteriaceae in Turkey

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Objectives: To evaluate the in vitro activity of ertapenem against *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. in the first year of its launch in Turkey.
Methods: A total of 416 non-duplicate clinical isolates of ESBL positive *E. coli*, ESBL positive *Klebsiella* spp. and *Enterobacter* spp. isolated during 2006 to 2008 from 5 University hospitals were included in the study. Antimicrobial susceptibilities of ertapenem and imipenem were determined by E-test method according to the CLSI breakpoints. ESBL production was confirmed at the coordinating centre by CLSI disk method by using both ceftazidime and cefotaxime with and without

clavulanate. A difference of ⩾ 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 carbapenem MIC of ⩾ 1 μg/ml were screened for carbapenemases 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 carbapenems 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 susceptibility. Eight OXA-48 and 1 VIM genes were found in 9 isolates, all of them were the modified Hodge test positive isolates. No IMP or KPC was found.

Conclusion: The susceptibility of ertapenem was 1–2% less than imipenem in ESBL producing Enterobacteriaceae. Isolates with putative carbapenemases were rarely encountered. The rest of the isolates with reduced sensitivity to carbapenems most 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

	MIC_{50}	MIC_{90}	Range	Susceptibility (%)
E. coli ESBL(+), n = 166				
Ertapenem	0.064	0.25	0.012–24	99
Imipenem	0.19	0.25	0.125–1.5	100
Klebsiella spp. ESBL(+), n = 162				
Ertapenem	0.094	0.38	0.008–32	97
Imipenem	0.19	0.25	0.125–32	99
Enterobacter spp., n = 88				
Ertapenem	0.047	0.5	0.008–32	97
Imipenem	0.25	0.38	0.125–32	99

P1041 Colistin resistance in multidrug-resistant Gram-negative bacteria

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Objectives: Colistin is considered as a last-resort therapeutic option for Gram-negative bacteria. The aim of the present study was to examine the susceptibility pattern of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* to colistin.

Methods: During a three-year period from 01/11/05 to 31/10/08 a total of 1836 strains of *Acinetobacter baumannii*, 1772 strains of *Pseudomonas aeruginosa* and 1245 strains of *Klebsiella pneumoniae* were tested for susceptibility to colistin among other agents. Isolates were recovered from blood cultures, catheters, pus, bronchial secretions and urine samples. Susceptibility testing of colistin was performed via the Vitek II system (Biomerieux, France) and confirmed through E-test strips (AB Biodisc, Sweden), according to the CLSI guidelines.

Results: *Acinetobacter baumannii* was resistant to colistin up to 1.5% (1.1% for the first twelvemonth, 2.3% for the second and 1.22% for the third one). *Klebsiella pneumoniae* isolates showed resistance to colistin at 16% (20% for the first twelvemonth, 12.6% for the second and 14.7 for the third one). *Pseudomonas aeruginosa*'s resistance to colistin was at 4.8% (8.2% for the first twelvemonth, 4.9% for the second and 1.3% for the third one). Colistin resistant strains were also resistant to most other antimicrobial agents, namely β-lactams, quinolones and aminoglycosides.

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.

P1042 Molecular mechanisms of resistance to rifaximin in "in vitro" selected enterotoxigenic and enteroaggregative *Escherichia coli* mutants

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Objective: The main aim of this study was to analyse the ole of efflux pump and point mutations in the rpoB gene in the development of rifaximin (Rfx) resistance in *Escherichia coli*.

Methods: *Bacterial strains*: Parental *E. coli* (2 enterotoxigenic and 2 enteroaggregative) were isolated from patients with traveller's diarrhoea in the Hospital Clínic of Barcelona. Fifteen Rfx 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 G-3′ and 5′-GCT TAT CAG CAC GCA GAG TCG GAA-3′, 30 cycles to 94ºC during 30″, 60ºC during 30″ and 72ºC during 30″, final elongation 72ºC 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 suggests that some of them (512, 525) do not play a relevant role (Table).

Strain	Initial MIC	Inhibitor effect	Amino acids	
			position	change
23233 parental	16	–	–	–
19769 parental	16	–	–	–
19768 parental	32	–	–	–
21835 parental	4	–	–	–
19768–32*	128	8×	–	–
19768–64*	>128	⩾16×	526	H → N
19768–128*	>128	–	516	D → N
19769–16*	>128	⩾16×	525	T → R
19769–64*	>128	–	516	D → N
19769–32*	>128	⩾8×	525	T → R
19769–128*	>128	⩾16×	512	S → F
21835–16*	>128	⩾8×	–	–
21835–32*	>128	⩾4×	574	S → Y
21835–64*	>128	–	516	D → G
21835–128*	>128	–	526	H → L
23233–16*	>128	⩾4×	516	D → G
23233–32*	>128	⩾4×	516	D → G
23233–64*	>128	⩾4×	516	D → N
23233–128*	>128	⩾4×	516	D → G

*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 PAbetaN 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.

P1043 In vitro activity of azithromycin against qnr carrying *Salmonella enterica*

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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 $\geqslant$0.125 μg/ml) but being susceptible or only low-level resistant to nalidixic acid (MIC $\leqslant$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 $\geqslant$32 μg/ml) was detected in 17 (2.8%) and high-level resistance (MIC $\geqslant$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

P1044 Clinical and analytical studies of a real-time PCR assay for the detection of toxigenic *Clostridium difficile*

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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. diff was developed and prospectively tested at three clinical sites.
Methods: Prodesse's ProGastro Cd assay, targeting the toxin B gene (tcdB) consists of a three-step process including Stool Clarification, nucleic acid extraction on the NucliSENS 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). Discrepant results were resolved via a combination of sequencing, culture, and EIA analyses.
Results: The ProGastro Cd Assay has a limit of detection of 1×10^3 CFU/mL of bacteria in clinical matrix and is able to detect from 5 to 5×10^9 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.

ProGastro Cd assay	Clinical Truth*			
	Positive	Negative	Total	
Positive	102	1	103	Sensitivity 96.2% (90.7–98.5%) 95% CI
Negative	4	664	668	Specificity 99.8% (99.2–100%) 95% CI
Total	106	665	771	

*Numbers recalculated after completion of Discrepant Analysis.

P1045 Real-time PCR assays for the detection of *Clostridium difficile* ribotype 017 and 027 strains

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Objective: *Clostridium difficile* is the causative agent of antibiotic-associated diarrhoea 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 PCR 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, was 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 toxinogenic *Clostridium difficile* strains in human faeces

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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 10^1 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 faeces 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 optimalisation of the toxB PCR resulted in the use of 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 analyzed by reculturing faeces samples and retesting DNA samples by toxB PCR. We were able to culture toxinogenic *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

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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% verses CCAT) may be less sensitive compared to culture. We aimed to compare the sensitivity and specificity of the Premier toxin 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 Hammersmith 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 1. Comparison of *C. difficile* EIA and RT-PCR with culture positive and toxin positive results[a]

Assay	Result	Comparison to culture positive + toxin positive results					
		No. of specimens		Sens	Spec	PPV	NPV
		Pos	Neg	(%)	(%)	(%)	(%)
Premier toxins A&B	Positive	7	1	36.8	99.2	87.5	91.4
	Negative	12	127				
Xpert™	Positive	18	6	94.7	95.3	75.0	99.2
	Negative	1	122				
PROGASTRO™	Positive	11	4	91.7	95.0	73.3	98.7
	Negative	1	76				

[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 MLPA-oligochromatographic multiplex assay for identification of the toxin genotype and the hypervirulent ribotype 027 of *Clostridium difficile*

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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 toxinogenic 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 O27 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 I) 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 (24 h and 48 h) of 5 strains were also tested successfully even without DNA extraction step. No cross-reactivity was observed with DNA extracted from negative faecal sample. DNA extracted from human faecal 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 faecal sample extraction to result) takes only 4 h30 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 epidemiological interest of the test relies on its easiness: one assay to detect 6 targets and only a 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 faecal specimens

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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 recurrency and therapeutic failures have highlighted the necessity of a rapid and reliable method for the detection of toxin-positive strains in faecal 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 aims were 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 faecal 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 specimens 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 EIA 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 toxinogenic 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.

P1050 Rapid oligochromatographic assay for the detection of group B *Streptococcus* in vaginal samples

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Objectives: To evaluate a rapid and simple new commercial assay (SpeedOligo GBS, Vircell) for the detection of group B *Streptococcus* (GBS) based on a PCR plus a rapid test device that provides results in less than one hour.
Methods: 235 double vaginal swabs were assayed. Bacteriological culture was carried out on Granada medium and Columbia blood sheep agar. Sterile swabs were artificially inoculated with GBS suspensions in order to determine the analytical sensitivity of the technique. DNA was extracted by direct heating and a fragment of cfb gene was amplified. Amplicons were detected by means of a dipstick in a 5 minutes hybridisation step: two complementary probes, one conjugated to gold particles and the other immobilised onto the membrane were used. The reaction was visually read. Discrepant results were reanalyzed by a qPCR based on sip gene.
Results: Culture was positive in 72 samples, 67 also positive by SpeedOligo GBS (93% sensitive vs. culture). The five false negative results corresponded to samples with <5 colonies in culture; SpeedOligo was able to detect the strains of these five samples when run on bacterial suspensions prepared from the agar plate. 13 additional samples were only detected by SpeedOligo GBS (92% specific vs. culture). 11 of these were confirmed by qPCR on a second target, giving SpeedOligo a final 99% specificity while an 87% sensitivity was calculated for culture. The analytical sensitivity of SpeedOligo was established in 225 microorganisms per swab, corresponding to 4 microorganisms per reaction.
Conclusion: SpeedOligo GBS proved to be a rapid, sensitive and specific assay for the detection of GBS colonisation, based on nucleic acid amplification techniques. The few false negative results were probably due to the low colonisation level of the patients, but the technique was able to detect the strains isolated from them. Some culture negative samples could be detected with the new assay and confirmed by an alternative qPCR test.

P1051 Characterisation of human herpes viruses and enteroviruses using a microarray technology: CLART® Entherpex

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Introduction: The Herpesviridae family viruses are widely spread among human population and they have the ability to establish lifelong latent infection. In immunocompetent individuals, the virus reactivation is usually harmless and unnoticeable. Human herpesviruses and enteroviruses are the major causative agents of the central nervous system (CNS) viral infections. The similar clinical signs provoked by these viruses make the diagnosis difficult and it is necessary to use diagnostic methods that allow multiple, sensitive, and rapid identification of these viruses.
Objective: To develop a high sensitivity and specificity system for simultaneous detection of Human Herpesviruses (HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7 and HHV-8) and Enteroviruses (Echoviruses, Poliovirus, Cosxackievirus), called CLART® Entherpex.
Method: Virus detection is performed via multiplex RT-PCR for amplification, and a new technology platform based on low-density micro-arrays (ArrayTube) for visualisation. This technology allows simultaneous detection of viruses and any necessary control in order to guarantee the reliability of the results obtained. In order to determine the diagnostic parameters of the kit, a comparative evaluation of CLART® Entherpex against clinical diagnostic's current methods (Herplex, PCR

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

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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. gallolyticus* (2), *S. infantarius* (1), *S. suis* (1), *S. cristatus* (1) and non-specified streptococci (6). Remaining cases included *Bartonella quintana* (2), *Bartonella* sp. (2), *Cardiobacterium* sp. (2), *Haemophilus* sp. (1), *Gemella haemolysans* (1), *Lactococcus garviae* (1), *Corynebacterium simulans* (1), *Propionibacterium acnes* (1), *Tropheryma 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 agens of IE from tissue samples to the species level, including differentiation of streptococci and detection of rare pathogens.

P1053 Use of a new and simple microarray assay (Prove-it™ Sepsis) for rapid identification of bacteria involved in bone and joint infections directly from clinical samples

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Objectives: The microbiological diagnosis of bone and joint infections (BJIs) currently relies on standard cultures, which are time consuming and are subject to false negative results in case of fastidious organisms and previous antibiotic therapy. Prove-itTM Sepsis (Mobidiag®) is a new commercial PCR and microarray assay that rapidly and reliably identifies more than 50 bacterial species present in positive blood cultures in less than 3 hours. The aim of our study was to evaluate the performance of this assay for the detection and identification of bacteria directly from BJI clinical samples compared to conventional culture and universal 16S rDNA PCR.
Methods: We selected 59 clinical samples (33 articular fluids and 26 biopsies), for which a microbiological diagnosis and identification had been established previously either by culture (C+) or PCR only (C-/PCR+). All these positive samples were chosen within the spectrum of bacteria included in the microarrays. Ten negative clinical samples (C-/PCR-) were also included. DNA was extracted with the automated MagNa Pure System (Roche) and the Prove-itTM assay was performed according to the manufacturer's protocol initially dedicated to blood culture. A human β-globin PCR was used to control DNA extraction and absence of inhibitors.
Results:

- C–/PCR– (n = 10): all samples were negative by using Prove-ItTM assay,
- C+ (n = 10): 7 samples were positive with concordant identification, whereas 3 remained negative (*S. epidermidis*, *S. aureus*, and mixed culture *S. aureus+P. acnes*),
- C–/PCR+ (n = 49):
  - 34 samples (69.4%) were positive with concordant identification with the following bacteria: *S. aureus* (n = 10), *S. epidermidis* (n = 10), *S. pyogenes* (n = 3), *S. agalactiae* (n = 3), *S. dysgalactiae* (n = 3), *K. pneumoniae* (n = 3), *S. marcescens* (n = 1) *P. aeruginosa* (n = 1),
  - 1 sample was positive indicating a mixture of 4 species, of which only one was identified by universal 16S PCR,
  - 14 samples were negative which corresponded to the following diagnoses by 16S rDNA PCR: 4 belonging to *Staphylococcus* sp, 7 to *Streptococcus* sp, 2 Enterobacteriaceae and 1 anaerobe.

Conclusion: These promising preliminary results indicate that this new microarray method allows a rapid and reliable detection of bacteria involved in BJIs. Providing additional probes relevant in BJIs (such as *Kingella kingae*, *Propionibacterium acnes*) being included in the array, it could replace conventional 16S rDNA PCR for clinical samples from patient suspect of BJIs.

P1054 Detection of OXA carbapenemase production in clinical samples by the Hyplex®CarbOxa ID multiplex PCR-ELISA system

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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 *Acinetobacter* strains producing the class D carbapenemases (mainly oxacillinases), is of great concern for the international community, since carbapenems 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 imipenem 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

specificity of the hyplex®CarbOxa ID method for blood culture and bronchial secretions samples were 100%.
Conclusions: The hyplex®CarbOxa ID test can be reliably applied for the rapid detection of blaOXA carriage by *A. baumannii* isolates in positive blood cultures and bronchial secretions in this setting. However, the efficiency of the test should also be evaluated in other clinical specimens, since it has the potentials of a useful diagnostic tool.

P1055 Identification of TEM-type extended-spectrum β-lactamases genes based on real-time PCR and pyrosequencing

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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 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). The presence of ESBLs were confirmed by standard microbiological techniques (disk diffusion antibiogram, synergy testing) and by standard PCR followed by sequencing.
Results: A 400-bp DNA fragment was amplified containing the four known positions (104, 164, 238–240) conferring an ESBL-phenotype. Ten well characterised ESBLs and broad-spectrum TEM-enzymes were unambiguously identified by pyrosequencing the four DNA regions of 15 bp responsible for extension of the profile. With one Light Cycler PCR reaction (20 μl), the four regions could be sequenced. Furthermore, the PCR was very sensitive, since 102–103 copies of blaTEM genes could be reproducibly detected. When bacterial isolates contained both TEM-1 and a TEM-ESBL derivative, pyrosequencing could easily distinguish between the two variants, and unambiguously indicate the presence of an ESBL. This high throughput technique has identified two TEM-ESBLs among the 20 clinical ESBL-producing *E. coli* isolates tested. All contained in addition a broad spectrum TEM-1-like variant. The other 18 ESBLs were of CTXM-type.
Conclusions: The combination of real-time PCR with pyrosequencing represents a powerful tool for epidemiological studies of TEM-producers. This assay is of high throughput since up to 96 bacterial isolates may be screened in less than 3 h. Furthermore, it allows to detect TEM-ESBLs even in presence of TEM-1/2 enzymes.

P1056 Multiplex real-time PCR detection of plasmid-mediated AmpC

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Objectives: Plasmid-mediated AmpC (pAmpC) confer resistance to penicillins, cephamycins, oxyimino-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 cloxacillin and boronic 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 pAmpC 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.

P1057 Rapid detection and identification of zygomycetes in clinical samples using high-resolution melt analysis

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Objectives: Invasive fungal infections are life-threatening complication in haematooncological 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 Zygomycetes targeting 18S region of ribosomal DNA. We modified 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 (Cotbett Research).

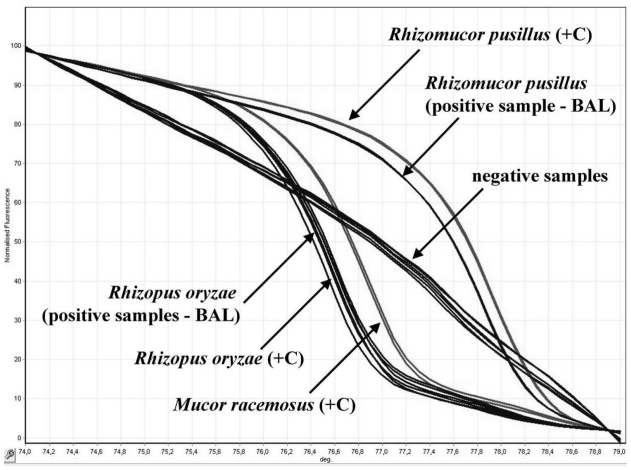

Figure 1. High Resolution Melt (HRM) analysis. +C: positive control.

Results: We tested 83 samples from 53 haemato-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., *Cunninghamella* 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

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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 presence of protoscoleces microscopically and were preserved in 70% ethanol at −20ºC. 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 sequnced 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 tho the same genotype.

P1059 Seroreactivity of dense granule recombinant antigens in toxoplasmosis

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Objectives: *Toxoplasma gondii* is an ubiquitous parasite that infects a broad range of hosts, including man and domestic animals. Toxoplasmosis in immunocompetent adults is usually asymptomatic. In contrast, primary infection during pregnancy can be transmitted to the fetus throughout the placenta, causing spontaneous abortion or severe fetal abnormalities. This fact emphasizes the importance of being able to make an accurate distinction between primary infection and reactivation. Among the available commercial diagnostic tests, serology is commonly used. The specificity and sensitivity of these serological methods depend mostly on diagnostic antigen(s) used. Most of these commercial kits use a native parasite antigen prepared from tachyzoites. Recombinant proteins would be alternative sources of antigens. An advantage would be the reduced test costs due to the lower costs of production and purification of recombinant antigens. In the present study, we have evaluated the usefulness of the *T. gondii* eight dense granule recombinant antigens (GRA) in diagnostic tests.
Methods: The gra genes were prepared by PCR amplification using the total tachyzoite DNA from RH *T. gondii* strain as a template. The PCR products were cloned into pUET1 vector, which appended six histidine codons to the 3′ and 5′ ends. The recombinant antigens were produced in the form of fusion proteins in *Escherichia coli* expression system and were purified by a one-step metal affinity chromatography. The immunoreactivity of the purified antigens was confirmed by ELISA with sera from patients with acute and chronic infection.
Results: Analysis of the results showed, that the sensitivity of in-house IgG rec-ELISA test performed with particular recombinant antigens or their mixture was 24% to 100% depending on the antigen used and the groups of examined sera. Specificity of IgG rec-ELISA was 100%. Moreover, r-GRA2, r-GRA7 and r-GRA8 proteins were much more correlated with acute toxoplasmosis than the chronic one, while r-GRA1 and r-GRA6 antigens detect antibodies in both groups of examined sera with the same sensitivity.
Conclusion: The applied expression systems allow for production of immunologically active *T. gondii* GRA recombinant proteins. These antigens are useful for serodiagnosis of toxoplasmosis and in the near future can replace the crude Toxoplasma antigens.

P1060 A real-time PCR for the detection of *Plasmodium ovale* variant strains

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Objectives: 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 Po and *P. vivax* (Pv). Sequence variations in 18S-rDNA lead to describe a classic and a variant type of Po (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 resulting 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

a prompt and targeted therapy with positive impact on the clinical management of the patients.

P1061 Detection of aetiology of peritonitis in peritoneal dialysis patients by PCR

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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 primerility 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, gelE. 3 samples carried *S. mutans* strains with the putative virulence genes PA, PGP, PGPK. 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.

P1062 Comparative analysis of serum proteomes to discover biomarker for scrub typhus

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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 2,300 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 PRO2044, PRO2675, apolipoprotein E, albumin-like protein, glutathione peroxidase, WFDC5, 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 Fc 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.

P1063 Molecular profile of rotavirus in children hospitalised with acute gastroenteritis in Norway, 2006–2008

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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%, 60% 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.

P1064 Comparison of two matrix-assisted laser desorption ionisation-time of flight mass spectrometry systems for rapid identification of bacteria and yeasts in a clinical laboratory

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Introduction: Matrix-Assisted Laser Desorption Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS) allows an identification of bacteria and yeasts within minutes generating species and genus specific

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
Enterococci (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, 6 (19%) G
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.

P1065 Bacterial species identification directly from urine samples by MALDI-TOF MS fingerprinting

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Objectives: The potential of MALDI-TOF MS to rapidly identify and distinguish various bacteria has been demonstrated in recent studies. Examination of biological fluids plays a certain role in clinical tests and recently several studies have addressed the issue of urine proteome. Urine represents the most easily attainable specimen and therefore one of the most usable in clinical diagnostics. Thus the application of MALDI-TOF MS fingerprinting through urine seems feeding in clinical laboratory diagnosis.
Methods: Fresh urine (15 ml) was centrifuged at 2500 rpm for 20 minutes at 37ºC. The supernatant was removed. Suitable for mass spectrometry water (1 ml) was added to the sediment, resuspended thoroughly and heated at 37ºC for 20 minutes, followed by a further centrifugation step. Then 300 µl of water was added to the sediment. After precipitation with ethanol (900 µl) and centrifugation, the pellet was suspended in 10 µL of pure acetonitrile, 10 µl 70% formic acid, and analyzed in a microflexTM (Bruker Daltonics, Germany). All clinical urine specimens were tested in parallel by both traditional microbiological methods according to standard laboratory protocols (WHO, ASM) and MALDI TOF MS combined with pattern recognition analysis (MALDI Biotyper 2.0; Bruker Daltonics, Germany).
Results: Overall, 200 urine samples from individual patients were studied. For 179/200 specimens (89.5%) the testing data were identical by both standard laboratory methods and MALDI Biotyper. In other cases discordance between the MALDI Biotyper and bacteriological testing was found. For 4 specimens (3.8%) the presence of bacteria was revealed by both methods, but the species ID were different. The correctness of MALDI identification was confirmed by 16S rRNA sequencing. Five samples were successfully tested by MALDI Biotyper that was confirmed by sequencing too while bacteriological tests did fail (no growth). For 12 samples (6%) MALDI-TOF MS analysis gave no identification results. The most cases of failure were closely associated with the titre of microorganism in original urine. Clinical significant titre of microbes is more then 104–105 CFU/ml that is congruent with the sensitivity threshold of direct bacterial profiling.
Conclusion: The MALDI-TOF MS direct bacterial fingerprinting from urine samples looks very attractive for species identification avoiding the step of cultivation.

P1066 Dermafinder: a new approach for fast and sensitive detection of dermatophyte skin infections

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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.

Antimicrobial resistance in Gram-positive bacteria

P1067 Long-term effects of the pneumococcal conjugate vaccine on antimicrobial resistance in children

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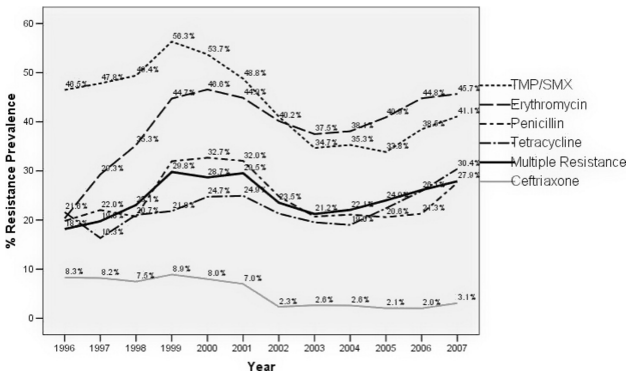
Objectives: To model the changes in *S. pneumoniae* resistance to several antimicrobial classes five years before and six years after the introduction of the conjugate pneumococcal vaccine in the US.
Methods: A total of 129,562 isolates from the TSN Network® surveillance database (Eurofins Medinet) during the period 1996 to 2007, as well as age, specimen source, inpatient or outpatient status and US census region were available for analysis. To appropriately model the rise,

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.



Resistance prevalence (%) in <5 year olds.

P1068 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)

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Background: Belgium is a country with one 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 diagnostic 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: 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).

FQ	MIC distribution				EUCAST			Efflux		
	geom. mean		MIC_{90}		Bkpt	%S		max	% Efflux for	
	−R	+R	−R	+R		−R	+R	(dil ×)	1 dil°	⩾2 dil°
LVX	0.83	0.71	1	1	2	97.8	98.5	1	3.7	0
MXF	0.16	0.14	0.25	0.25	0.5	98.5	98.5	1	27.6	0
GRN	0.04	0.03	0.06	0.06				2	19.4	0.7
GEM	0.03	0.01	0.06	0.03				3	83.6	17.2

P1069 Penicillin resistance in Scottish invasive *Streptococcus pneumoniae* isolates, between 1999 and 2007

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Objectives: *Streptococcus pneumoniae* remains a major cause of bacteraemia 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 μg/ml), and 7 (0.15%), were penicillin resistant, (MIC > 1 μg/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 μg/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 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.
Due to poor drug penetration into the CSF, clinical treatment failure of pneumococcal meningitis with penicillin may be seen with MIC ⩾ 0.12. Interestingly, 171 (3.6%) of these isolates were from CSF, and 6, (3.5%), were non-susceptible to penicillin. Five serogroups/types were identified, of which one (serotype 3), is not covered by the 7-valent conjugate vaccine. Three sequence types were identified (156, 442 and 180).
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.

P1070 Regional variations in penicillin resistance rates against *Streptococcus pneumoniae*: The United States, 2008

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Background: The percentage rates of penicillin-resistant (PenR) *S. pneumoniae* (SPN) varies 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. MIC's 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 PenI rates in most regions. Regional changes from a 1999–2000 study to 2004–2008 study are noted. Tigecycline and vancomycin had the lowest MIC90 s (mcg/mL) against PenR SPN at 0.5 followed by LEV and LNZ at 1 and CFX and AC at 2 and 8, respectively.
Conclusions: PenNS for SPN has remained essentially constant since 1999, but PenR has generally shifted from Pacific regions eastward. VAN, LNZ, LEV and TIG MIC90 values remain unaffected by pen phenotypes.

Regions	Pen I+R 1999–2000,% n = 4,751	Pen I+R 2004–2008,% n = 1,694	Net Gain (Loss),%
East South Central	53.3	60.3	7.0
South Atlantic	47.9	47.9	0
West North Central	37.1	45.7	8.6
West South Central	47.5	45.1	(2.4)
Middle Atlantic	36.9	39.7	2.8
East North Central	38.7	39.6	0.9
Mountain	41.1	36.3	(4.8)
Pacific	34.6	32.3	(2.3)
New England	26.1	32.3	6.2
All Regions	41.0	42.4	1.4

P1071 Trends in antimicrobial resistance in *Streptococcus pneumoniae*, Canada, 1993–2008

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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 slightly between 2006 and 2007 (19.3 vs 22.4 and 11.2 vs 13.1 respectively). Preliminary results from 2008 suggest a decreasing or stable trend in 2008. Resistance to fluoroquinolones declined 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 (meningeal breakpoint MIC $\geqslant$ 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.

	'93	'94	'95	'96	'97	'98	'99	'00	'01	'02	'03	'04	'05	'06	'07	'08
	5.7	8.1	8.8	11.9	13.6	15.0	13.4	12.3	14.4	15.1	14.9	14.8	15.3	15.4	17.2	16.0
Pen R	0.9	1.3	2.2	4.1	6.6	5.7	5.9	5.9	6.8	6.5	6.2	5.4	4.8	6.2	4.6	6.0
Amox R	0	0	0	0	0.1	0.1	0.2	0.1	0.5	0.6	1.1	1.1	1.1	1.4	1.6	1.9
Eryth R	1.9	3.4	3.2	4.8	6.8	10.5	9.8	11.2	12.8	14.0	15.9	18.0	19.1	19.3	22.4	21.2
Clinda R	0	1.7	1.3	2.4	3.6	5.1	4.4	5.5	5.8	6.5	7.4	8.1	8.2	8.6	9.6	7.9
T/S R	3.8	4.7	9.7	12.7	14.6	12.3	12.0	11.3	11.9	13.1	13.3	13.5	12.2	11.7	11.9	10.9
Tet R	1.4	2.3	3.4	2.5	6.4	9.1	7.1	5.5	9.1	9.6	9.7	10.9	10.4	11.2	13.1	9.2
Ceftrx R(M)	0	0.2	0.1	0.7	1.3	2.5	1.5	2.0	2.4	1.5	1.8	2.5	1.9	3.7	2.9	3.3
Ceftrx R(NM)	0	0.04	0	0.2	0.1	0.1	0.3	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.3	0.2
Cipro R	0.5	0.8	0.7	0.8	1.8	1.8	1.6	1.4	2.1	2.6	1.7	2.0	2.4	2.7	2.0	2.2
Levo R	0	0.4	0.1	0.2	0.5	0.3	0.4	0.9	1.2	1.8	1.2	1.5	1.5	1.7	1.2	1.6
Moxi R	NT	NT	0	0	0.3	0.2	0.2	0.4	0.4	0.3	0.4	0.6	0.7	0.9	0.7	0.7

P1072 Resistance and resistance mechanisms in *S. pneumoniae* in the Netherlands the Duem3 study

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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 study. 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. MIC's 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 >1 mg/L for Le, and >0.5 mg/L for Cl. EUCAST susceptibility criteria were used.
Results: 583 strains were tested, of which 542 were available and confirmed SP after reidentification. MIC90 s 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 ermB type and 41% due to efflux (mefA and mefE) showing a similar pattern as earlier surveys. 0.6% was Le R 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 cotrimoxazole.

P1073 Macrolide resistance in *Streptococcus pneumoniae* from the T.E.S.T. Program in Europe: a multi-year analysis

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Background: Even with decreases in antibiotic consumption, 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 Europe against macrolide-resistant *S. pneumoniae* during the years of 2004 to 2008.
Methods: 459 clinical isolates were collected from 61 investigative sites from 19 countries in the 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 the 121 (26.5%) 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 would be effective against this important clinical pathogen and resistant phenotype.

Drug	Macrolide-resistant *S. pneumoniae* (n = 121)			
	MIC_{50}	MIC_{90}	% Susceptible	% Resistant
Tigecycline	0.03	0.12	na	na
AmoxClav	=0.03	2	97.5	0.8
Cefdinir	0.25	>2	62.8	36.4
Ceftriaxone	0.06	1	86	0
Cefuroxime	0.25	8	63.6	34.7
Erythromycin	64	>64	0	100
Levofloxacin	0.5	1	100	0
Linezolid	=0.5	1	100	0
Minocycline	2	8	33.9	62
Moxifloxacin	0.12	0.25	98.3	1.7
Penicillin	0.12	2	47.9	13.2
TrimethSulfa	0.5	>4	62	31.4

na = breakpoints not defined.

P1074 Macrolide resistance in *Streptococcus pneumoniae* in the United States and Canada, 2004–2008

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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.

Drug	Macrolide-resistant *S. pneumoniae* (n = 371)			
	MIC_{50}	MIC_{90}	% Susceptible	% Resistant
Tigecycline	0.03	0.12	na	na
AmoxClav	0.5	4	78.7	7.3
Erythromycin	64	>64	0	100
Cefdinir	2	>2	40.2	54.7
Ceftriaxone	0.25	1	90.3	3
Cefuroxime	2	8	39.4	57.4
Levofloxacin	0.5	1	98.7	1.1
Moxifloxacin	0.12	0.25	98.9	1.1
Linezolid	=0.5	1	100	0
Minocycline	0.5	8	0.5	0.5
Penicillin	0.5	4	18.9	37.5
TrimethSulfa	4	>4	24	62.8

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

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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 10/18. MIC90 values of tigecycline remained unchanged with $\leqslant$0.125, 0.25, 0.5, $\leqslant$0.125 and $\leqslant$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

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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/sulfamethoxazole and fusidic acid retained high in vitro activity against

nosocomial *S. aureus* strains in Russia. Resistance to fluoroquinolones, lincosamides, macrolides, aminoglycosides, tetracyclines, fusidic acid, rifampicin and chloramphenicol substantially increased during five years period.

Antibiotic	2001–2002 (n = 879)			2006–2007 (n = 577)		
	MIC_{50} (mg/l)	MIC_{90} (mg/l)	I+R (%)	MIC_{50} (mg/l)	MIC_{90} (mg/l)	I+R (%)
Chloramphenicol	8	128	42.9	64	64	57.0
Ciprofloxacin	0.5	4	13.0	16	64	54.4
Clindamycin	0.125	256	27.1	0.06	512	43.0
Erythromycin	0.5	256	39.5	1	512	50.8
Fusidic acid	0.125	0.25	0.0	0.06	0.06	2.4
Gentamicin	0.5	256	30.6	64	256	53.2
Levofloxacin	0.25	1	9.0	4	16	52.8
Linezolid	2	2	0.0	1	2	0.0
Mupirocin	0.25	0.25	0.3	0.125	0.25	0.9
Rifampicin	0.03	0.03	6.9	0.015	256	24.8
Tetracycline	0.5	128	37.0	0.5	64	41.9
Co-trimoxazole*	0.125	0.5	0.8	0.06	0.25	0.17
Vancomycin	1	1	0.0	1	1	0.0

*MIC values are indicated for trimethoprim.

P1077 Susceptibility of *Staphylococcus aureus* in community settings: first Russian surveillance

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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.

Antibiotic	MIC_{50}	MIC_{90}	S (%)	I (%)	R (%)
Chloramphenicol	8	64	73.6	0	26.4
Ciprofloxacin	0.5	1	93.3	1.9	4.8
Clindamycin	0.06	0.125	95.4	0	4.6
Erythromycin	0.25	512	81.8	1.0	17.2
Fusidic acid	0.06	0.125	100	0	0
Gentamicin	0.5	1	96.3	0	3.8
Levofloxacin	0.125	0.25	96.2	0	3.8
Linezolid	2	2	100	0	0
Mupirocin	0.125	0.25	100	0	0
Netilmicin	0.25	0.5	99.5	0.2	0.3
Oxacillin	0.5	0.5	96.2	0	3.8
Rifampicin	0.015	0.015	98.8	0	1.2
Tetracycline	0.5	32	84.4	0.2	15.3
Co-trimoxazole*	0.06	0.06	100	0	0
Vancomycin	1	1	100	0	0

*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.

P1078 Community-associated methicillin resistant *Staphylococcus aureus*: a retrospective epidemiological study of cases reported in Canton Ticino, Switzerland, between 2002 and 2008

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Objectives: To characterise the clinical and microbiological epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) in Southern Switzerland (Canton Ticino).
Methods: We analyzed all cases of MRSA reported to the Cantonal Institute of Microbiology in Bellinzona between 1.1.2002 and 31.12.2008. CA-MRSA were defined on the basis of their susceptibility to ciprofloxacin; resistance to gentamycin, tetracycline or to the combination of sulfamethoxazole and trimethoprim (SMX/TMP); and production of Panton-Valentine leukocidin (PVL). The CA-MRSA strains were analyzed by spa-typing and for the presence of the cassette chromosome (SCCmec) alleles IV and V. An epidemiological definition for CA-MRSA (CDC criteria) was also used if epidemiological and clinical data were available.
Results: During the study period 1793 MRSA strains were isolated. 250 (13.9%) were classified as CA-MRSA. The number of CA-MRSA isolates per year remained virtually unchanged (average: 35). Ciprofloxacin-resistant strains decreased over time from 69.7% in 2002 to 36.1% in 2008, while resistance to tetracycline and SMX/TMP tended to increase (tetracycline: 15.1% in 2002, 27.8% in 2008; SMX/TMP: 9.1% in 2002, 27.8% in 2008). Resistance to gentamycin appeared to decline (70% in 2002 vs. 25% in 2008). In the preliminary analysis, CA-MRSA carriers defined by epidemiological criteria were typically young (median age 32.5, range 20–52), predominantly female (62.5%), and without significant co-morbidities. Patients presented with skin, soft tissue or ocular infections; no case of necrotising pneumonia was observed. 83.3% of the CA-MRSA with available epidemiological and clinical information were PVL positive.
Conclusions: In Southern Switzerland the number of newly identified CA-MRSA strains per year has remained unchanged over the last 5 years. The isolates were mostly sensitive to several classes of antibiotics, but we observed an increase of CA-MRSA strains resistant to tetracycline and SMX/TMP. On the contrary, there was a decrease in the resistance to ciprofloxacin and gentamycin.

New antimicrobials against Gram-positives

P1079 Comparison of bacterial membrane active novel porphyrin and metalloporphyrin antimicrobials

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Objectives: XF-70, XF-73 and DPD-207 are novel porphyrin drug candidates which display rapid bactericidal activity against *S. aureus*. It has previously been demonstrated that the lethal anti-staphylococcal activity of XF-73 (a non-metalloporphyrin) is due to its rapid effects on the integrity of the cytoplasmic membrane. This study sought to compare and contrast the mode of action of XF-73 with XF-70 (a further non-metallo porphyrin) and DPD-207 (an iron metalloporphyrin).
Methods: The integrity of the *S. aureus* cytoplasmic membrane after exposure to lethal concentrations of XF-70, XF-73 and DPD-207 and control agents was determined using a range of techniques. Membrane potential was measured using a DiSC3(5) fluorimetric assay, leakage of intracellular potassium and magnesium was determined by atomic absorption spectroscopy and a luciferin/luciferase assay was used to quantify the leakage of intracellular ATP. The morphology of cells after

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-207. 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

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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 with reduced susceptibility to glycopeptides.
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), 0.12/2 mcg/mL against strains from Asia (n = 97) and 0.12/1 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.

Agent	Number of strains	MIC range	MIC_{50}	MIC_{90}
DFX	961	⩽0.004–16	0.12	0.5
LVX	961	0.03–>32	8	>32
LNZ	961	⩽0.25–2	1	1
DAP	946	0.25–4	1	1
VAN	961	⩽0.25–2	0.5	0.5
CLI	961	⩽0.03–>8	0.12	>8
ERY	961	⩽0.12–>16	>16	>16
TIG	961	⩽0.015–0.5	0.06	0.12
OXA	961	4–>8	>8	>8

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

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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 SASPject, 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) (10^7 cfu/ml final concentration) were incubated with PT1.2 (1.5×10^8 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 37ºC 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 ⩾4-$\log_{10}$ 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

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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; VRS1 – fully vancomycin-resistant and GP06 – teicoplanin-intermediate. Spontaneous resistance to LTX was assessed by subculture on agar plates in the presence of 2×, 4× and 8× 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 sub-culturing 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).

Strain	MIC (mg/L)			
	FUS		LTX	
	P0	P14	P0	P14
29213	0.06	512	2	4
433300	0.06	64	2	4
VRS1	0.06	32	1	8
Mu50	0.03	16	1	2
GP06	1	128	2	8

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

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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 ug/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

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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 92.5%, 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

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Objectives: MUT056399 is a novel antimicrobial agent targeting the essential enzyme FabI which catalyzes 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: IC50 s 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 13nM. MUT056399 displayed an antibacterial spectrum consistent with specific FabI inhibition: a high potency against methicillin susceptible and resistant staphylococci (mode MIC $\leqslant 0.06$ 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 10^4 to 10^6 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 antistaphylococcal 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.

These properties support MUT056399 as a very promising new chemical entity for clinical development as a new drug to treat staphylococcal infections.

P1086 Farnesol as a prospective antimicrobial agent against *Staphylococcus epidermidis*

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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.

P1087 The potential of the breakdown products of casein by *Lactococcus lactis* strain 146 as an inhibitory therapeutic agent(s) for MRSA

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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 diaries 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.

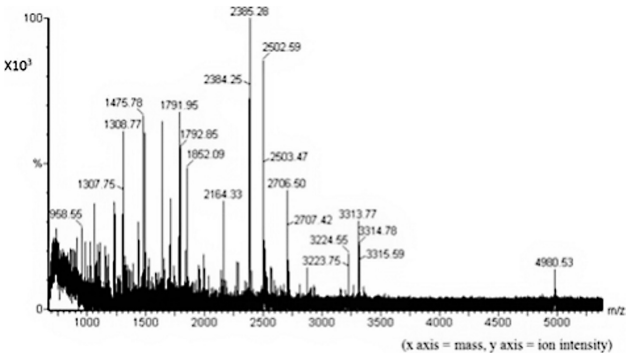

Figure 1. Detection of peptide species of casein breakdown products by Lactococcus lactis strain 146 using mass spectrometry. Resulting amino acids were detected on a time-of-flight (TOF) mass spectrometer with matrix-assisted-laser-desorption ionisation (MALDI).

P1088 The potential of the naturally produced inhibitory substance (A345) as a potential therapeutic agent for treating *Staphylococcus aureus*

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Objectives: In recent years, there has been much focus on a promising class of bacteriocins known as lantibiotics. The most prominent representative of lantibiotics, nisin, has already a long history of use in the protection of foodstuffs. Lantibiotics have also been considered for application in humans; however they have not yet been used in the setting of chemotherapy on the same scale as traditional antibiotics.
We here investigate the cationic peptide antibiotic (A345) as a future opportunity for treating staphylococcal infections.
Methods: 1. Investigation of *S. aureus* strain A345 was performed using simultaneous and deferred-antagonism against MRSA.
3. Purification and/or concentration of free inhibitors in broth supernatants was carried out using ammonium-sulphate precipitation, Sep-Pak® cartridge, Speed-Vac®, Cation-exchange; then C18 reverse-phase chromatography.
6. MALDI TOF/TOF was used for mass analysis.
7. Electron microscopy was used for ultra-structure diagnosis.

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).

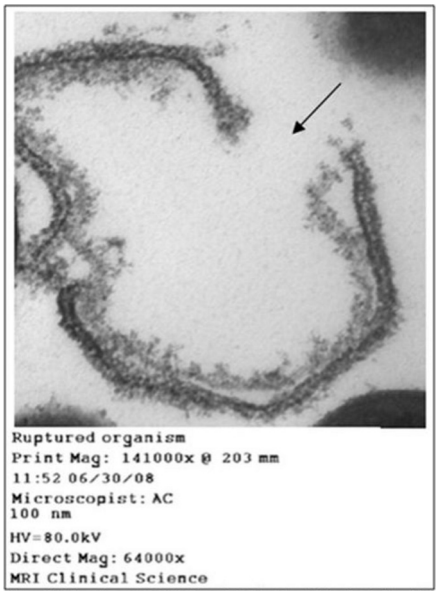

Figure 1. Thin section of EMRSA-15 strain A208 cell wall after incubation with the inhibitory substance A345 for 24 h at 37ºC. An example of a ruptured wall is seen.

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 cationic-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.

P1089 The safety of multiple ascending oral doses of TR-701, a novel oxazolidinone prodrug antibiotic

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Objectives: TR-701, a novel oxazolidinone prodrug antibiotic of the microbiologically-active moiety TR-700 is primarily active against Gram-positive organisms. A randomised, double-blind, placebo- and active-controlled, 21-day oral dose study of TR-701 was performed.
Methods: 40 healthy male and female subjects were enrolled in cohorts of 10 subjects each (8 active and 2 placebo), and received 21-days of single oral doses of 200, 300, or 400 mg TR-701, or 600 mg linezolid twice-daily. Both drugs were administered under fasting conditions. Safety was assessed via adverse events (AEs), physical examination, ECG and laboratory evaluations. Reporting of AEs was solicited proactively in subjects confined for 25 days.
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.

Summary of subjects with adverse events after multiple ascending oral doses of TR-701

	Number of Subjects (%)				
	Placebo	TR-701 QD			Linezolid
		200 mg	300 mg	400 mg	600 mg BID
	n = 8	n = 8	n = 8	n = 8	n = 8
Any AE within 21 days	5 (62.5)	5 (62.5)	5 (62.5)	7 (87.5)	4 (50)
Mild	5 (62.5)	5 (62.5)	5 (62.5)	7 (87.5)	4 (50)
Moderate	1 (12.5)	–	2 (25)	3 (37.5)	1 (12.5)
Severe	–	–	–	–	–
Treatment-related AEs					
during 21 days of drug administration	4 (50)	4 (50)	4 (50)	7 (87.5)	4 (50)
during the first 7 days of drug administration	2 (25)	–	1 (12.5)	5 (62.5)	2 (25)
AEs leading to study discontinuation	–	1 (12.5)	–	2 (25)	1 (12.5)
Serious AEs	–	–	–	–	–

P1090 The safety of single ascending oral doses of TR-701, a novel oxazolidinone prodrug antibiotic

P. Bien, K.A. Munoz, P. Prokocimer, J. Bohn (San Diego, Madison, US)*

Objectives: The prodrug TR-701 is 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

	Number of Subjects with AEs (%)[a]						
	Placebo	TR-701					
		200 mg	400 mg	600 mg	800 mg	1200 mg	Overall
	n = 10	n = 6	n = 6	n = 6	n = 6	n = 6	n = 30
Any AE[b]	0	4 (66.7)	2 (33.3)	3 (50.0)	1 (16.7)	3 (50.0)	13 (43.3)
Treatment-related AEs	0	3 (50.0)	0	3 (50.0)	1 (16.7)	3 (50.0)	10 (33.3)

[a] Each subject with any AE(s) is counted once.
[b] All events were reported as "mild".

Results: 28 treatment-emergent AEs were reported by 13 subjects receiving TR-701 and no AEs were reported by subjects receiving placebo. 19 AEs were considered treatment-related. All AEs were considered "mild" in severity. There were no apparent dose-related trends in AE reporting. Similar numbers of AEs were reported in each cohort with the greatest number occurring following administration of 200 mg TR-701. AEs reported in at least 2 subjects receiving TR-701 were:

nausea (3 subjects [10.0%]), dizziness, diarrhoea, and nasal congestion (2 subjects each [6.7%]). There were no deaths, Serious AEs, or discontinuations due to AEs. No clinically significant changes or findings were noted in 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.

P1091 Effects of food on the pharmacokinetics of TR-701, a novel oxazolidinone prodrug antibiotic, in healthy adult subjects

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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-hr fast or after eating a high-fat breakfast preceded by a 10-hr 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 AUC0-t and AUC0-inf 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. There were no SAEs or deaths during this study and no AE led to study discontinuation.
Conclusions: Single oral doses of 600 mg TR-701 administered in the fed and fasted state were well tolerated. Administration of TR-701 after a high-fat meal increased Tmax and decreased Cmax, but did not affect the extent of TR-700 exposure (AUC). Since AUC is the main PK/pharmacodynamic (PD) driver of efficacy for oxazolidinones, the present study provides evidence that TR-701 can be administered regardless of the timing of meals.

P1092 TR-700, a novel methyltetrazolyl-oxazolidinone, accumulates extensively within human macrophages cells and shows activity towards intraphagocytic linezolid-sensitive and linezolid-resistant *S. aureus*

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Background: Treatment of intracellular infections requires that antibiotics reach their intracellular target and express activity therein. Linezolid accumulates poorly within cells, and shows only modest intracellular activity against *S. aureus* or *S. epidermidis* (Barcia-Macay et al, AAC, 2006; Pascual et al, AAC, 2002). The aim of this study was to examine the cellular pharmacokinetic properties and intracellular activity of TR-700 towards *S. aureus*, in light of the higher lipophilicity and intrinsic activity of this molecule vs linezolid.
Methods: Human THP-1 macrophages were used throughout this study. Accumulation of both oxazolidinones was measured by microbiological assay, using *S. aureus* ATCC 25923 as test organism. The phenotypes of the strains used are shown in Table 1. MICs were determined in MHB. Intracellular activity was determined over a large range of extracellular concentrations (typically 0.01 to 100-fold the MIC to obtain full description of concentration-effect relationships) against bacteria phagocytised by human THP-1 macrophages (see details in Barcia-Macay et al, AAC, 2006). Results are expressed here as (i) bacteriostatic concentration (SC), and (b) maximal effect (Emax) at 24 h compared to time 0 h (post-phagocytosis) as calculated from the Hill functions fitted to the data by non-linear regression.
Results: TR-700 accumulated quickly and extensively in macrophages, within 15 minutes reaching an apparent cellular to extracellular concentration ratio of about 13 vs 1–2 for linezolid. MICs in broth (mg/L) and intracellular activities are shown in Table 1.

Organisms	Linezolid			TR-700		
	MIC	intracell. activity		MIC	intracell. activity	
	(broth)	SC[a]	E_{max}[b]	(broth)	SC_a	E_{max}[b]
ATCC 25923	2	~4.5	−0.4±0.1	0.25	~1.0	−0.6±0.1
SA 238[c]	2	~5.8	−0.3±0.1	0.25–0.5	~0.5	−0.7±0.1
SA 238[c]	16	>100*	+0.2±0.1	1	~1.0	−0.6±0.1
CM-05[d]	8	~21.3	−0.5±0.3	0.25–0.5	~0.7	−0.6±0.1

[a]Extracellular antibiotic concentration (mg/L) yielding no apparent change in cfu after 24 h compared to post-phagocytosis inoculum.
[b]Maximal decrease in intracellular cfu (log scale) compared to the post-phagocytosis inoculum.
[c]Strains selected in the laboratory, [d]linezolid-resistant MRSA clinical isolate carrying the *cfr* methyltransferase gene.
*Bacterial growth in all conditions.

Conclusions: Compared to linezolid, TR-700 shows increased potency (lower bacteriostatic concentrations) towards intraphagocytic *S. aureus* (unaffected by resistance of the strain to linezolid), probably in relation with its extensive accumulation within cells and its higher intrinsic activity (lower MIC values).

P1093 Ceftaroline activity tested against common organisms causing skin and skin-structure infections in European medical centres during 2008

R. Jones, G. Moet, H. Sader (North Liberty, US)*

Objectives: To assess the activity of ceftaroline and comparator agents tested against SSSI pathogens. Ceftaroline is the bioactive metabolite of ceftaroline fosamil, a N-phosphonoamino water-soluble cephalosporin prodrug. Ceftaroline is active against methicillin-resistant *S. aureus* (MRSA) and other resistant pathogens and is under evaluation for treatment of skin and skin-structure infections (SSSI) in clinical trials.
Methods: Unique (1 per patient) clinically significant isolates of *S. aureus* (2168), β-haemolytic streptococci (BHS; 172), viridians 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 ceftriaxone (CRO), linezolid (LZD) and vancomycin (VAN), respectively. The highest ceftaroline MIC among MSSA was 1 mg/L, and 90.9 and 99.8% 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 MRSA 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.)	MIC90 (mg/L)/% Susceptible					
	Ceftaroline	CRO	LEV	CLI	LZD	VAN
MSSA (1617)	0.25/NA	4/99.8	⩽0.5/94.6	⩽0.25/98.0	2/100.0	1/100.0
MRSA (551)	2/NA	>32/0.0	>4/14.5	>2/64.3	2/100.0	1/100.0
BHS (172)	0.015/NA	⩽0.25/100.0	⩽0.25/100.0	1/90.7	1/100.0	0.5/100.0
VGS (86)	0.25/NA	0.5/90.7	⩽0.25/97.7	2/88.4	1/100.0	0.5/100.0
E. faecalis (409)	8/NA	>32/NA	>2/71.4	>4/NA	2/100.0	2/98.8

NA = not assigned.

P1094 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 for *S. aureus* PBP 2a and demonstrated bactericidal activity against methicillin-resistant *S. aureus* (MRSA) and other pathogens responsible for CARTI.

Methods: CARTI isolates (717) were consecutively collected in 2008 from 24 hospitals located in 10 European countries (EU), Turkey, and Israel. *S. aureus* isolates were obtained from patients with pneumonia occurring less than 72 hours after hospitalisation. Susceptibility (S) was tested by CLSI broth microdilution method against ceftaroline and various antimicrobials used to treat CARTI.

Results: The potency of ceftaroline against 3 common pathogens associated with CARTI is summarised in the Table.

Organism (no. tested)	Cumulative % inhibited at ceftaroline MIC (mg/L) of:									
	⩽0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4
PEN-S SPN* (328)	85.1	97.0	98.5	99.7	99.7	100.0	–	–	–	–
PEN-intermediate SPN* (41)	4.9	19.5	53.7	87.8	97.6	100.0	–	–	–	–
PEN-R SPN* (78)	0.0	1.3	2.6	6.4	71.8	98.7	100.0	–	–	–
Beta-lactamase-neg HI (170)	86.5	99.4	99.4	100.0	–	–	–	–	–	–
Beta-lactamase-pos HI (29)	65.5	96.6	100.0	–	–	–	–	–	–	–
MSSA (47)	0.0	0.0	0.0	0.0	0.0	85.1	100.0	–	–	–
MRSA (24)	0.0	0.0	0.0	0.0	0.0	0.0	29.2	75.0	91.7	100.0

*Penicillin breakpoints of ⩽0.06 and ⩾2 mg/L were applied.

Against *S. pneumoniae* (SPN), the activity of ceftaroline (MIC50/90, ⩽0.008/0.12 mg/L) was eight-, 16-, and 64-fold more potent than ceftriaxone (MIC50/90, ⩽0.25/1 mg/L), amoxicillin/clavulanate (A/C; MIC50/90, ⩽1/2 mg/L), and cefuroxime (MIC50/90, ⩽1/8 mg/L), respectively. Penicillin (PEN) resistance (R) was high among SPN; only 73.4% and 92.0% of strains were inhibited at ⩽0.06 and ⩽2 mg/L, respectively, whereas A/C inhibited 93.3% of strains at ⩽2 mg/L. R was also high among the SPN isolates for erythromycin (33.6%), azithromycin (33.1%), tetracycline (27.5%), clindamycin (21.7%), and trimethoprim/sulfamethoxazole (17.0%). Ceftaroline was very active against *H. influenzae* (HI; MIC50/90, ⩽0.008/0.015 mg/L), and its activity was not adversely affected by β-lactamase production. Ceftaroline (MIC50/90, 0.25/0.5 mg/L) was eight- to 16-fold more potent than ceftriaxone (MIC50 and MIC90, 4 mg/L) and cefepime (MIC50/90, 2/4 mg/L) against methicillin-susceptible *S. aureus* (MSSA). The highest ceftaroline MIC value among MRSA was 4 mg/L (only 2 strains), and 75.0% of all isolates were inhibited at ⩽1 mg/L of ceftaroline. All non-MRSA Gram-positive cocci were ceftaroline susceptible at ⩽0.5 mg/L.

Conclusions: Bacterial 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.

P1095 Spectrum and potency of ceftobiprole against leading North American pathogens producing community- and hospital-acquired pneumonia (2005–2007)

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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. Against PSA, 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 cephems but including MRSA. These characteristics warrant continued evaluation of BPR as empiric therapy for treating bacterial pneumonia.

Organism (no. tested)	BPR MIC (mg/L)		Cum. % inhibited at MIC (mg/L)					
	50%	90%	⩽0.25	0.5	1	2	4	8
Community-acquired								
H. influenzae (HI; 883)	⩽0.06	⩽0.06	100.0	–	–	–	–	–
S. pneumoniae (SPN; 1912)	⩽0.06	0.5	88.9	98.5	100.0	–	–	–
Hospital-acquired								
S. aureus (SA; 938)	0.5	2	26.7	54.2	89.3	100.0	–	–
P. aeruginosa (PSA; 492)	4	>8	1.2	5.7	25.0	45.1	60.2	77.9
Klebsiella spp. (KSP; 228)	⩽0.06	>8	75.9	77.6	79.4	80.3	81.1	82.5
Enterobacter spp. (EBS; 155)	⩽0.06	8	75.5	78.1	80.7	85.8	89.7	91.6
Acinetobacter spp. (ASP; 122)	>8	>8	10.7	21.3	30.3	33.6	36.1	36.1

P1096 Antimicrobial activity of ceftobiprole, a novel anti-methicillin-resistant *S. aureus* cephalosporin, tested against skin and skin-structure infection pathogens (North America)

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Objectives: To establish ceftobiprole (BPR) activity for this investigational parenteral cephalosporin under regulatory review for complicated skin and skin structure infections (SSSI). 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 SSSI pathogens in North America (NA) are presented.

Methods: Non-duplicate clinically-significant SSSI isolates (1,466) were collected from over 25 medical centres in NA participating in a BPR

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 % 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 recognized activity against NA SSSI pathogens, similar to that of extended-spectrum cephems but including MRSA. These characteristics warrant continued evaluation of BPR as empiric therapy for SSSI, including Gram-negative pathogens.

Species (no. tested)	MIC_{90} in mg/L (% at ⩽2/⩽4/⩽8 mg/L)		
	BPR	CRO[a] or CAZ[b]	FEP
S. aureus (SA; 896)	1 (100/–/–)	>32 (15/49/51)[a]	>16 (40/57/76)
P. aeruginosa (PSA; 100)	8 (67/79/92)	>16 (66/83/87)[b]	8 (60/76/90)
E. coli (EC; 99)	⩽0.06 (96/97/97)	⩽1 (97/98/98)[b]	0.25 (98/98/98)
E. faecalis (EF; 60)	1 (100/–/–)	–	–
Beta-haemolytic streptococcus (BHS, 52)	⩽0.06 (100/–/–)	⩽0.25 (100/–/–)[a]	⩽0.12 (100/–/–)
Enterobacter spp. (ESP, 54)	8 (83/89/93)	>16 (63/65/70)[b]	4 (89/96/98)
Klebsiella spp. (KSP; 42)	>8 (83/83/83)	>16 (86/88/88)[b]	2 (90/90/90)
P. mirabilis (31)	⩽0.06 (100/–/–)	⩽1 (97/100/–)[b]	⩽0.12 (100/–/–)

CRO = ceftriaxone, CAZ = ceftazidime, FEP = cefepime.

P1097 In vitro activity of ceftobiprole against a group of well-characterised staphylococci

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Objectives: In an effort to test the in vitro activity profile and the antibacterial spectrum of ceftobiprole, 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 ceftobiprole (BPR) and the main anti Gram-positive antimicrobials (vancomycin, teicoplanin, daptomycin, linezolid, synercid, 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 stains); 8 CA-MRSA; 26 MSSA 20 MRCoNS, and 6 control strains.
Results: Ceftobiprole had a MIC90 value of 4 mg/L against the MDR Iberian, Rome and Italian 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 the sporadic and MSSA isolates included in the study. In vitro activity of ceftobiprole 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), synercid (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: Ceftobiprole 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 microrganisms.

P1098 Efficacy and pharmacodynamic evaluation of CEM-101, a novel macrolide, in murine infection models

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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 *S. 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 efficacy 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 0.59 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 r^2=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

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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 AUC24 h similar to that of telithromycin 800 mg qD 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-I and 7 TEL-R based on EUCAST breakpoints [S ⩽ 0.25 – R > 0.5]). MICs were determined by geometric microdilution 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.

Phenotype*	No.	TEL			CEM-101		
		range	geom. mean	MIC_{90}	range	geom. mean	MIC_{90}
TEL-S	16	0.008–0.25	0.021	0.25	0.008–0.063	0.022	0.063
TEL-I	6	0.5–0.5	0.5	0.5	0.063–0.5	0.223	0.5
TEL-R	7	1–3	1.426	3.0	0.5–1.0	0.906	1.0

*MLS_{β} for 7/16 of TEL-S, 5/6 of TEL-I, and 7/7 of TEL-R isolates
(S/I/R are defined based on EUCAST breakpoints (S ⩽ 0.25 − R > 0.5).

P1100 Antimicrobial characterisation of CEM-101: activity against staphylococci, β-haemolytic and viridans group streptococci

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Objectives: To address therapy of MLSB-resistant (R) species, CEM-101 (a new macrolide-ketolide), was developed with enhanced potency against wildtype (WT) respiratory tract (RTI) and cutaneous (SSSI) pathogens. Results of CEM-101 susceptibility (S) testing against 452 staphylococci and selected streptococci are described here.
Methods: A collection of 2006–2007 clinical isolates were S tested by CLSI methods (M7-A7) with associated interpretive criteria (M100-S18) and supplements (2–5% LHB) for streptococcal tests. CEM-101, telithromycin (TEL) and 10 comparators were used versus 201 *S. aureus* (75 WT-MRSA, 75 WT-MSSA, 30 CA-MRSA, 17 VISA or hVISA, 7 VRSA), 100 coagulase-negative staphylococci (CoNS; 10 species), 100 β-haemolytic (BHS; 30 group A, 31 group B, 14 group C, 9 group F, 16 group G) and 51 viridans group streptococci (VGS; 5 species), see Table.
Results: MSSA strains were slightly more CEM-101-S (MIC50, 0.06 mg/L) that MRSA or CA-MRSA strains (MIC50, 0.12 mg/L). VISA, hVISA and VRSA were generally more refractory to CEM-101 and TEL. CEM-101 was 2-fold more potent than TEL against all staphylococci. Streptococci were very S to CEM-101 (MIC90, 0.03–0.06 mg/L) and TEL was 4-fold less active with non-S isolates of BHS observed. ERY-R staphylococci remained CEM-101-S except for TEL- and clindamycin (CC)-R isolates, but all BHS and VGS were S to CEM-101.
Conclusions: CEM-101, a novel macrolide-ketolide, was potent against all staphylococci (MIC50, 0.06 mg/L), except CC-R strains; and inhibited all streptococci at ⩽0.12 mg/L. The activity was greater than TEL by 2- to 4-fold. CEM-101 warrants further development for RTI and SSSI indications.

Organisms (no.)	CEM-101 MIC (mg/L)			Telithromycin MIC (mg/L)		
	50%	90%	Range	50%	90%	Range
MSSA (75)	0.06	0.12	0.03–>16	0.12	0.25	0 06–>16
MRSA (75)	0.12	>16	0.03–>16	0.25	>16	0.06–>16
CA-MRSA (30)	0.12	0.12	0.06–0.12	0.25	0.25	0.12–0.5
VISA, hVISA (14)	>16	>16	0.06–>16	>16	>16	0.25–>16
VRSA (7)	>16	–	0.12–>16	>16	–	0.12–>16
CoNS (100)	0.06	>16	0.03–16	0.12	>16	0.03–>16
BHS (100)	0.015	0.03	⩽0.008–0.12	0.03	0.12	⩽0.008–2
VGS (51)	⩽0.008	0.06	⩽0.008–0.12	0.015	0.25	⩽0.008–0.5

P1101 Activity of CEM-101 tested against emerging telithromycin-resistant β-haemolytic streptococci

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Objectives: To assess CEM-101, a macrolide-ketolide in early pre-clinical development, potency against strains observed to be resistant (R) to other agents in the same class. Reports have documented ketolide-R (telithromycin [TEL]) species worldwide, most recently *S. pyogenes* from Europe. 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 MLS_{β}-ketolide comparisons agents

Antimicrobial	Occurrences at MIC (mg/L):									
	⩽0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	>4
CEM-101	4	0	1	18	10	6	4	0	0	0
Telithromycin	0	0	0	0	0	0	0	8	16	19
Erythromycin	0	0	0	0	0	0	0	0	–	43
Clindamycin	–	–	–	–	2	1	0	0	–	40
Q/D[a]	–	–	–	–	37	6	0	0	–	0

[a]Q/D = quinupristin/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 at ⩽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.

P1102 Antibacterial activity of NXL103 (linopristine-flopristine), in vitro post-antibiotic effect, and spontaneous frequency of resistance

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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 (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 (37ºC, 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^{-9} to 1.8×10^{-10} at

concentrations of 2 and 4×MIC (MIC increased from 0.25 to 2–4 mg/L). No mutants were isolated at 8×MIC (2 mg/L) (frequency $<2.9\times10^{-10}$). 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 mg/L, respectively) (frequency $<3.1\times10^{-10}$).
Conclusion: 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

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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 37ºC on Mueller-Hinton agar (MHA) or in liquid broth (MHB). MIC values were determined via microdilution in accordance with CLSI guidelines. Serial passage experiments were carried out by plating ~1×10^{8} CFU on TR-700 and LZD antibiotic gradient plates. Following a 48 h incubation, the leading edge of growth was streaked into MHB without antibiotics and grown overnight for the subsequent passage plating. 23S rRNA mutations were determined through sequencing the domain V region of all 23S rRNA gene copies for each strain.
Results: After 20 serial passages, TR-700-passaged MRSA 33591 had an MIC increase from 0.25 to 0.5 ug/mL, but MSSA 29213 and LMRSA CM-05 TR-700 MIC values did not change. LZD resistance for MSSA 29213 and MRSA 33591 increased from 2 to 32 ug/mL and 0.5 to 4 ug/mL, respectively, while remaining unchanged for LMRSA CM-05. 23S rRNA domain V sequencing of passage 20 cultures revealed mutations only in strains with elevated MIC levels. MRSA 33591 passaged in TR-700 possessed a novel dual T2571C/G2576T mutation in 2 of the 23S rRNA genes. LZD-passaged MSSA 29213 had 3 copies of the G2447T mutation and LZD-passaged MRSA 33591 had 2 copies of the G2576T mutation.
Conclusion: The ability to evolve antibiotic resistance over time and the underlying resistance mechanisms are important measures in assessing the risk of emerging resistance for an antibiotic compound. Through serial passage we have shown that *S. aureus* has a very limited ability to develop elevated levels of resistance to TR-700. Further analysis of the novel coupled T2571C/G2576T mutations found in TR-700-passaged MRSA 33591 may shed light on the low frequency of resistance to TR-700.

P1104 Characterisation of the novel oxazolidinone TR-700 and linezolid spontaneous mutation frequencies and resistance mechanisms in *Staphylococcus aureus*

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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 37ºC 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 $<1.2\times10^{-10}$ and $<2.0\times10^{-10}$, 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.
Conclusion: Spontaneous mutation frequencies 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 over 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

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Background: Ceftaroline (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) 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

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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 nº 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>MIC (h)] were calculated administering single doses of 20 mg/kg/ip of UB, CIP and MOX to groups of 21 uninfected animals. To study the in vivo efficacy of UB, a 72 h murine pneumonia model was performed; bacteria were inoculated intratracheally (8.72±0.11 log10 cfu/mL for Ab58, and 8.44±0.10 log10 cfu/mL for Ab33), and groups of 15 mice were randomly ascribed to non treated (control), and treated with 20 mg/kg of UB, CIP, or MOX every 8 hours. Analyzed variables: bacterial lung concentration (Log10 cfu/g), negative blood cultures (%), and mortality (%). 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>MIC (h) were 0.94, 1.40, and 1.47. PD parameters for Ab33 (UB, CIP, and MOX): AUC/MIC were 6.55, 1.10, and 3.56; T>MIC (h) were 0.89, 0.73, and 1.14. Results of the pneumonia model are in the table.
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.

Treatment	Ab58				Ab33			
	AUC/MIC 0–24 h	Bacterial lung concentration (log_{10} cfu/g)	Sterile blood cultures (%)	Mortality (%)	AUC/MIC 0–24 h	Bacterial lung concentration (log_{10} cfu/g)	Sterile blood cultures (%)	Mortality (%)
Control	–	8.86±0.94	26.6%	80%	–	8.12±1.09	18.8%	68.8%
UB	327.54	4.24±2.49*	86.7%*	53.3%	19.65	3.97±3.32*	81.3%*	37.5%
CIP	105.48	2.67±2.83*	80.0%*	26.6%*	3.30	6.57±1.99	60.0%*	66.7%
MOX	668.13	2.98±2.42*	86.7%*	33.3%*	10.68	4.64±2.26*	76.5%*	47.1%

*$p < 0.05$ respect to control group.

P1107 Activity of PZ-601 (SMP-601) against Enterobacteriaceae with AmpC, ESBLs and carbapenemases

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Background: PZ-601 (SMP-601) is a novel 1 β-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 clinical *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 ertapenem-susceptible ESBL-positive *Klebsiella* spp. was 2 mg/L (range 0.25–8 mg/L), with little change for ertapenem-resistant produces lacking porins. The mode MIC for ESBL-positive *Enterobacter* spp. was 8 mg/L (range 1–32 mg/L); that for AmpC-derepressed *Enterobacter* was 8 mg/L (range 4–16 mg/L) rising to 16 mg/L (range 16–>128 mg/L) where porin loss and ertapenem 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

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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 [PIP/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 were clinical successes vs. 35.5% (11/31) of COMP-treated subjects (weighted difference 25.9; 95% CI 3.7%, 48.0%). 17 subjects in each group received adjunctive amikacin, gentamicin, ciprofloxacin, or tobramycin with clinical success in 47.1%(8/17) of DORI vs. 23.5% (4/17) of COMP ($p = 0.282$) subjects that received adjunctive therapy. Pooled clinical success rates for each disease were as follows: cUTI, 100%(11/11) DORI vs. 0%(0/1) LVX; cIAI, 100% (2/2) DORI vs. 100% (3/3) MER; NP/VAP, 45.2% (14/31) DORI vs. 29.6% (8/27) (IMI and PIP/TAZO). Adverse events were reported in 36 (78.3%) DORI and in 29 (85.3%) COMP-treated subjects.
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

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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 in the ME at TOC 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) $\leqslant$4 mg/L 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 $\leqslant$4 mg/L 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 $\leqslant$4 mg/L vs susceptible comparators (81.4% vs 67.6%; 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.

P1110 Resistance development studies with the novel siderophore monobactam BAL30072

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Objective: Multi-resistant clinical isolates of Gram-negative bacilli were investigated for their propensity to develop resistance to the novel monobactam antibiotic BAL30072.
Methods: Isolates of *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Stenotrophomonas maltophilia*, *Enterobacter cloacae* and *Klebsiella pneumoniae* were examined for their propensity to spontaneously acquire resistance to BAL30072, and selected comparators, either after single-step selection or through repeated passaging in cultures with increasing concentrations of test substance.
Results: The single step resistance frequencies were in the range 10^{-10} to 10^{-8} for BAL30072 against strains of Enterobacteriaceae and *Acinetobacter* spp. The frequency of appearance of spontaneous resistance to ceftazidime or meropenem in susceptible strains was between 10^{-9} and 10^{-6}. Subpopulations with elevated MICs against BAL30072 were identified in all *P. aeruginosa* strains, with incidence between 10^{-9} and 10^{-6}. Subpopulations resistant to ceftazidime or meropenem were also detected at an incidence between 10^{-7} and 10^{-4}. Repeated passaging in cultures containing BAL30072 yielded strains with <4-fold elevated MIC after 5–20 passages. Strains with <4-fold elevated MIC towards cefepime of meropenem appeared within 3–5 passages under the same conditions.
Conclusions: Low frequencies of spontaneous resistance to BAL30072 were observed and it was more difficult to select for resistant mutants in serial passage than it was for cefepime or meropenem.

P1111 Bactericidal effect of the novel siderophore monobactam BAL30072

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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 MBC of BAL30072 was 1–4×MIC against most strains of Enterobacteriaceae and *Acinetobacter* spp. tested. The MBC could be up to 16×MIC against some strains of *Pseudomonas aeruginosa* that were tested. Against the same strains the MBCs of cefepime 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 5×10^4 cfu/ml than for an inoculum of 10^7 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 BAL 30072 against ESBL-producing Enterobacteriaceae and clinical isolates of *Enterobacter cloacae*

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Objectives: BAL 30072 (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 strains 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); cefepime (CFEP). MICs were determined by CLSI methods but Muller-Hinton agar supplemented with 2.2 dipyridyl was used for BAL to induce iron transport.
Results: MICs, range, MIC50, MIC90 (mg/L) are shown in the table.

		BAL	MER	CTAZ	P/T	CFEP
ESBL producers						
E. coli	range	0.03–⩾64	0.008–0.06	2–⩾64	4–64	0.12–64
	MIC_{50}	1	0.015	16	32	8
	MIC_{90}	2	0.03	32	64	32
K. pneumoniae	range	0.06–⩾64	0.015–0.06	2–⩾64	32–64	0.25–8
	MIC_{50}	64	0.015	⩾64	32	2
	MIC_{90}	⩾64	0.06	⩾64	64	8
Ent. aerogenes	range	0.06–⩾64	0.015–0.06	0.12–⩾64	4–⩾64	0.03–8
	MIC_{50}	0.5	0.03	32	32	0.25
Clinical strains						
Ent. cloacae	range	0.008–⩾64	0.008–⩾0.25	0.12–⩾64	1–⩾64	0.015–16
	MIC_{50}	2	0.015	4	16	0.12
	MIC_{90}	⩾64	0.06	⩾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

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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 2 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 to KBPA 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.

P1114 Developing DNA-based therapies to tackle antibiotic resistance

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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 Decoys (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.

P1115 SASP: A novel antibacterial protein with potential to limit the spread of antibiotic resistance

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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 16 h incubation at 37ºC 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 (3×10^8 cfu) of *S. aureus* carrying pSM198 was infected with 6×10^8 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 16 h incubation at 37ºC 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.

P1116 In vitro susceptibility of *Campylobacter jejuni* to organic acids and monoacylglycerols

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Objective: Antimicrobial resistance among foodborne pathogens has been recognized as an important emerging public health problem. *Campylobacter* 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, capric, 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

glutamic acid. The antimicrobial activity was measured at two pH regimes (5.5 and 6.5) and was expressed as IC50, i.e. as the concentration of a tested acid that causes a 50% reduction of bacterial growth, in comparison with control (untreated) cultures. Effect of most effective organic acids on strain *C. jejuni* CCM 6214 was studied by transmission electron microscopy.
Results: Caprylic acid followed by capric and lauric acid (i.e. straight-chain fatty acids with 8 to 12 carbon atoms, respectively), were found to be the most active in inhibiting the growth of *C. jejuni* (IC50 <0.1 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.
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P1117 A note on the effect of medium-chain fatty acids on arcobacters

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Objectives: The genus *Arcobacter*, formerly known as aerotolerant *Campylobacter*, is included in the family Campylobacteraceae. Up to the present time, six species have been characterised within the *Arcobacter* genus. Out of six *Arcobacter* species, four are considered as emerging foodborne pathogens: *Arcobacter butzleri*, *A. skirowii*, *A. cryaerophilus* and *A. cibarius*. 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 diarrhoeal 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: Antibacterial 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(-ºCt)) 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. The most sensitive was *A. skirrowii*, followed by *A. cryaerophilus*. The strongest antibacterial activity was observed in lauric acid (C12).
Conclusion: Our results showed that medium chain fatty acids have a strong potential as alternative antibacterial agents in combating with arcobacters.
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P1118 Antimicrobial and anti-pathogenic features of some new oxepins

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Objectives: The aim of the present study was to evaluate some newly synthesized new oxepins for their antimicrobial and anti-pathogenic features, respectively.
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 antimicrobial 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 inert 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 interferred 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%), lecithinase (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.

P1119 In vitro antifungal activity of corifungin, a new water-soluble polyene, against sensitive and resistant *Candida* species

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Objective: The antifungal activity of a new water soluble polyene, corifungin, was evaluated against clinical strains of *Candida* species.
Methods: In vitro evaluation of corifungin was performed and compared to amphotericin B deoxycholate (AmB) against 35 different strains of *Candida* which included *C. albicans* (5), *C. krusei* (5), *C. tropicalis* (5), *C. lusitaniae* (5), *C. glabrata* (5), *C. dubliniensis* (5). This included several *C. glabrata* strains with multi-drug resistance patterns. In-vitro evaluation was performed using the CLSI standards and read at 48 hrs.
Results: The mean minimal inhibitory concentration (MIC) of corifungin and AmB for *C. albicans* was 0.5 μg/ml and 0.5 μg/ml, respectively, for *C. tropicalis* 0.5 and 1 μg/ml, for *C. dubliniensis* 0.125 and 0.5 μg/ml, for *C. parapsilosis* 0.5 and 1 μg/ml, for *C. lusitaniae* 0.5 and 1.0 μg/ml, for *C. krusei* 2 and 1 μg/ml, and for *C. glabrata* 0.5 and 1.0 μg/ml, respectively.
Conclusion: The results indicate that corifungin demonstrated excellent in vitro antifungal activity with low MICs against the most common *Candida* species, with slightly less activity against *C. krusei*. In addition, corifungin also demonstrated good activity against the several multi-azole resistant *C. glabrata* species. Corifungin appears to be an excellent candidate for the treatment of candidal infections.

P1120 Intracellular activity of anidulafungin against *Candida* spp.

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Objectives: Anidulafungin is a new echinocandin antifungal agent. It has fungicidal activity against many *Candida* spp., including fluconazole-resistant, and fungistatic activity against other yeast and moulds such as *Aspergillus* spp. The intracellular activity of anidulafungin against

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 opsonised yeasts (PMN/yeast; 1/10) were incubated for 30 min at 37ºC. 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 Saboureaud 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

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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 using 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

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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 reinjected 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

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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 alfa-terpineol 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 colture cells, stained with acridine orange, were examined by a fluorescence light mycroscopy 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 cytoplasms 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 37ºC 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 vescicles.

Conclusion: These data suggest that TTO interferes with an early step of Influenza virus replicative cycle, after viral adsorption. Our results indicated that this compound could inhibit viral uncoating by an interference with acidification of intralysosomal compartment.

P1124 Bio-informatic prediction of herpes simplex virus type 1 latency-associated transcript microRNA and their mRNA neurotransmitter targets

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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 miRNA 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.wistar.upenn.edu/BayesSVMmiRNAfind/), was used to predict miRNAs generated from HSV-1 LAT mRNA and the RNAhybrid program (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/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 mRNAs 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.

P1125 In vitro activity of five antimicrobial peptides against colistin-susceptible and resistant *Acinetobacter* spp.

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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 Acinetobnacter. 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, cecropin P1, buforin, indolicidin, a-defensin for colistin-suspectible 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). ii. Cecropin 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.). Indolicidin presented the same activity against all three *Acinetobacter* spp..
Conclusions: 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.

P1126 Squalamine, a new antibiotic extracted from marine environment

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Objectives: Extensive use of antibiotics has raised a serious public health problem due to infections caused by multidrug-resistant bacterial pathogens. Consequently, there is a pressing need to develop new antibiotics to keep pace with bacterial resistance. Recently, a new aminosterol called squalamine [1] has been isolated from tissues of the dogfish shark, Squalus acanthias. This unusual natural product has attracted considerable interest because of its potent antimicrobial activities against a broad spectrum of microorganisms. [1]
Squalamine
Methods: The feasibility of obtaining large quantities of this steroidal antibiotic, from natural sources or by synthesis, appears questionable. In order to understand the structure-activity relatationships of such compounds, we have recently developed the synthesis [2] of a structural analogue of squalamine [2] mimicking not only its structure but also its antimicrobial properties.
Results: We have envisioned the possibility that these derivatives might be able to disrupt the organisation of the Gram-negative bacterial membranes. The antibacterial action manifested via permeabilisation of the outer membrane of *Escherichia coli* bacteria has been demonstrated. [3] On the other hand, a different mechanism of action of such derivatives towards Gram-positive bacteria has been determined by electron microscopy analysis and membrane depolarisation measurements and will be discussed.
Conclusion: Squalamine is a membrane-active molecule that targets the membrane integrity and consequently, its activity and mechanism of action corelates with the membrane lipid composition.

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P1127 Large antibacterial activity spectrum of aminosterols derivatives towards multidrug-resistant Gram-negative and Gram-positive bacteria from patients with cystic fibrosis

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Objectives: Resistance to antibiotics is a life-threatening danger with more severe impacts on fragilised populations like cystic fibrosis (CF) patients [1]. Squalamine and AminoSterol Derivatives (ASDs) have demonstrated interesting antimicrobial activities against several bacterial and fungal reference strains 2, 3. However, when it comes to resistant clinical strains the challenge is evidently more difficult.
Methods: Antimicrobial activities of squalamine [1] and a synthesized aminosterol [2] against multidrug-resistant bacterial and fungal clinical strains were investigated. 135 bacterial isolates were recovered from sputa of CF patients and 70 fungal strains composed of 50 filamentous fungi isolated from sputa of patients with respiratory disorders (including CF) and 20 non-filamentous bloodstream isolates.

Results: In the case of Gram-negative bacteria, MICs ranged from 2 to 128 mg/L. Mucoidity of *P. aeruginosa* strains and resistance to colistin correlated significantly with high MIC values. In contrast, compounds 1–2 appeared very active against various Gram-positive bacteria with highest MIC value of 4 mg/L. Concerning the antifungal activity, compound 2 appeared more active than squalamine against tested fungal strains as reflected by MIC values (1–4 mg/L vs. 8–32 mg/L, respectively).
Conclusions: In spite of correlating with colistin in activity against Gram-negative bacteria, tested compounds demonstrated surprising higher antibacterial effect against Gram-positive isolates naturally resistant to colistin indicating probably the presence of different mechanism of action against both group of bacteria. On the other hand, the antifungal activity was notably enhanced by modifying the aminosterol structure. Taken together, our results indicate that ASDs possess a broad antimicrobial spectrum against various bacterial and fungal clinical strains.

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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

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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.

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

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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.

P1130 Effectiveness of heptavalent pneumococcal conjugate vaccination on invasive pneumococcal disease one year after the introduction in the Danish childhood vaccination programme

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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 bacteraemia 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 (y), the mean incidence declined significantly from 54 to 23 per 100,000 in the pre- and post-PCV7 ($p < 0.005$). In children <2 y, 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

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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%), 22(2.8%). 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 Valencia 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

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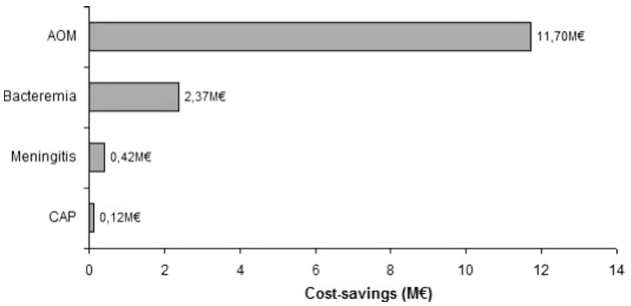
Objective: To estimate the total impact of infant vaccination with a 7-valent pneumococcal vaccine (PCV-7), a 10-valent PHiD-CV or a no-vaccination strategy across all ages in Finland.
Methods: The total vaccine impact is assessed with a 1-year, cross-sectional, overall population-based model at vaccine steady-state condition selecting local epidemiological and unit cost data. Direct vaccine effect is estimated as reduction in incidence and clinical outcomes associated with invasive diseases (ID) such as meningitis and bacteraemia, 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.



Cost-savings with PHiD-CV compound compared with PCV-7 by clinical outcome.

P1133 Serologic responses to revaccination with 23-valent pneumococcal polysaccharide vaccine among patients with HIV infection who received highly active antiretroviral therapy

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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

by ⩾2-fold from baselines when revaccination was administered were compared among the three groups of patients at 12th month of revaccination.
Results: At revaccination, patients of group 1 had a lower median CD4 count than those of the other two groups (325 vs.487 and 615 cells/μL, $P < 0.05$), while the percentage of undetectable HIV RNA load (<400 copies/ml by RT-PCR) was similar among the three groups (overall, 88.4%). Increases of CD4 count (overall, 27 cells/μL) and virologic responses to HAART (overall, 86.1%achieving undetectable HIV RNA load) 12 months after revaccination were similar among the three groups. At 12th month of revaccination, the overall proportion of responders to either one of the three serotype was 37.3% (38.2%, 40.0%, and 33.3% for groups 1, 2, and 3, respectively), while it was 23.7% to serotype 14, 12.5% to serotype 19F, and 14.8% to serotype 23F.
Conclusions: Five years following primary pneumococcal vaccination using 23-valent PPV, more than one third of the patients who continued to receive HAART responded to revaccination with 23-valent PPV at 12th month.

P1134 Antibody response to pneumococcal conjugate vaccination followed by pneumococcal polysaccharide vaccine in heart and lung transplant patients: a pilot study

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Objective: Steptococcus pneumoniae is a major cause of infections like pneumonia, and bacteraemia in transplant patients. At the time vaccination with the 23-valent pneumococcal polysaccharide vaccine (PPV23) is recommended for adult transplant patients.
However, the use of immunosuppressive medications may impair these patients‘ response to this polysaccharide vaccine. To enhance the immunogenicity of pneumococcal polysaccharides conjugate pneumococcal vaccines (PCV7) have been developed.
Therefore we conducted this pilot study to get an idea how effective the antibody production can be boostered by a pneumococcal polysaccharid vaccine after a prime-boost with a pneumococcal conjugate vaccine in heart and lung transplant patients.
Methods: 23 solid organ transplant adult patients visiting our outpatient clinic were enrolled in the study. 10 patients were in the lung transplant (LTX)- 13 in the heart transplant (HTX) group. All received an intradeltoid injection with 0.5 ml of a single lot of the 7-valent pneumococcal conjugate vaccine Prevenar® (Wyeth Pharma, GmbH). 8 weeks after the first application patients were vaccinated with the 23-valent polysaccharide vaccine (Pneumo 23 Vaccine Merieux®) which contains 23 serotypes. Blood samples for determination of pneumococcal antibodies were taken prior to each vaccination and 8 weeks afterwards. Serotype-specific IgG antibody concentrations to pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F were measured with a reference ELISA according to the WHO (World Health Organisation) protocol.
Results: Baseline anticapsular IgG concentrations were similar in both groups of patients. All HTX patients had an adequate antibody response, defined as a two-fold increase in specific IgG antibody titres, to the prime boost vaccination with PCV. However, the only difference to the LTX group was that these patients had no two fold increase in serotype 14 at week 8.
In week 8 patients were vaccinated with PPV23. None of the patients had a relevant increase in antibody titers measured at week 16.
Conclusion: PCV is immunogenic in adult heart- and lung transplant recipients. The patients however did not seem to benefit from a second PPV23 dose after a short time interval of 2 months. Thus, the optimal vaccine regime against pneumococcal disease has to be evaluated in further studies.

P1135 Circulating *Streptococcus pneumoniae*-specific effector B cells in humans in pneumococcal pneumonia and after pneumococcal polysaccharide vaccination

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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 pulmonar lymphocytes in humans. The data also show differences in the in the Ig-distribution of these cells after natural infection and pneumococcal vaccination.

P1136 Outpatient-based pneumococcal vaccine campaign and survey on perceptions about pneumococcal vaccination in patients and doctors

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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

P1137 20,000 hand-hygiene observations on 60 wards in 16 acute trusts: dispelling hand-hygiene myths? Or reporting a national change in practice?

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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)

[1] McAteer J et al, Development of an observational measure of healthcare worker hand-hygiene behaviour: the hand-hygiene observation tool (HHOT), Journal of Hospital Infection 2008; 68:222–229.

P1138 Production of the WHO-recommended alcohol-based handrub formulations in 11 different sites worldwide

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Objective: Availability of 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.

P1139 Successful implementation of the WHO multimodal hand hygiene improvement strategy and tools: a survey of 230 hospitals worldwide

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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.

Table

	Median score (range)*
Strategy element	
System change (ABHF, clean water and soap availability)	7 (4–7)
Staff education	7 (5–7)
Observation and feedback	7 (4–7)
Reminders	6 (3–7)
Promotion of a patient safety climate	6 (2–7)
Main tools	
Guide to implementation	6 (4–7)
Guide to local production	6 (6–6)
Educational tools	7 (6–7)
Manual for observers	7 (1–7)
WHO posters	6 (5–7)

*7-point Likert scale.

P1140 Impact of a campaign to improve hand hygiene in healthcare workers

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Objectives: To compare Health Care Workers (HCW)'s hands contamination and risk factors for before and after the implementation of a multimodal strategy to improve hand hygiene (HH) compliance in medical wards in an acute care university hospital.

Methods: A multimodal program to improve HH was conducted in October 2007. A total of 104 out of 132 HCW in medical wards were included and cultured in PRE (April to May 2007) and 89 in POST campaign periods (November to December); 89 hand paired samples of the same HCW were included. Cultures of the dominant hand were performed immediately preceding a patient care action during daily hospital practice. Hands were sampled by a glove juice method. Microorganisms identified were classified as resident flora, transient, and total flora (the sum of both). Data collected included: gender, professional category, skin condition, presence and number of rings, fingernail length, number of assigned patients, hours of work before sampling, self reported time of most recent hand washing episode and product used. Hand's bacterial loads were evaluated by comparing median values in each period.

Results: A total of 89 paired hands were cultured in both periods. 82% were female, 28% physicians, 38% nurses and 34% others. Resident flora was detected in 92.1% hands vs. 70.8% ($p = 0.001$) and transient flora in 96.6% vs. 75.3% ($p < 0.001$) in PRE and POST periods.

The hand median cfu counts decreased from 2782 (range 1–13747) to 1065 (range 0–13682) ($p < 0.001$), resident flora from 970 (range 0–12855) to 23 (range 0–13600) ($p < 0.001$), and transient flora from 10 (range 0–4935) to 2 (range 0–7323) ($p < 0.001$). Median cfu counts of Gram negative bacilli were 205.5 (range 0–4924) vs. 25.57 (range 0–831) ($p < 0.001$) in PRE and POST periods, respectively. No other significant differences were detected in transient flora.

In the POST intervention a lower number of rings ($p < 0.001$), shorter fingernail length ($p = 0.008$), shorter time of most recent hand washing ($p = 0.007$), and a most frequent use of alcohol solutions instead of soap ($p < 0.001$) were documented.

For the risk factors study a total of 193 sampled hands were included. In the linear regression model taking total flora as dependent variable, a longer time of most recent hand washing was associated with higher contamination rates (Beta=0.3, $p = 0.005$).

Conclusions: A HH multimodal strategy decreased the number of risk factors and the level of HCW hands contamination during daily hospital practice.

P1141 Impact of monitoring hand hygiene compliance during entire care episodes for measurement of compliance rates and interpretation of comparisons

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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, (v) and 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) (before or after a single contact, and before the first contact or after the last contact of a series of successive contacts) or as intra-series opportunities (ISO) (from the opportunity following 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%, $P < 10^{-6}$). 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.

P1142 Comparison of hand hygiene practices between physicians and nurses

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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.

Methods: Compliance, defined as hand washing/disinfection in the required setting, was directly observed by defined personnel in hospital wide during three months. The observed health care workers were not aware of this observation.

Results: A total of 661 hand hygiene opportunities were observed in 239 healthcare workers (109 physicians and 130 nurses). Mean compliance ratio was 80% and the ratios were 67.9% and 89.9% in physicians and nurses, respectively. According to these results compliance to good 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

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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 cultures of health care workers and the results of weekly consumption of AbHR.
Results: Compliance rates on IW are shown in the table.

Indications	Compliance with hand disinfection (%)					
	Nurses			Physicians		
	Baseline	After intervention	*p*	Baseline	After intervention	*p*
All indications[a]	75.9[c]	85.3[d]	<0.01	68.75[c]	63.9[d]	n.s.
Before patient contact	66.2	78.3	0.17	48.3	56.5	n.s.
Before invasive procedure	73.7	83.8	0.16	50	20	n.s.
After patient contact	92.9	95.8	n.s.[b]	83.8	82.6	n.s.
After contact with body secretions	69.4	86.9	<0.05	100	100	n.s.
After object	75	82.6	n.s.	50	25	n.s.
Between patients	84.2	83.3	n.s.	88.8	80	n.s.

[a] 417 observations during baseline period (week 1), 347 observations after intervention (week 6).
[b] n.s. = not significant; [c] difference not significant; [d] $p = 0.0004$.

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, on IW-B from 64 ml/patient day 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

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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 $p < 0.001$).
Conclusions: Our model shows 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

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Background: e-Bug is a DG SANCO funded antibiotic and hygiene teaching resource aiming to reinforces 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

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-Bug screenshot.

P1146 Association between the amount of hand antiseptics used and hospital-acquired infections in a Danish hospital

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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

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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

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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 (Hibi Liquid Hand Rub+) and antimicrobial soap hand wash formulation (Hibiscrub®) 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 Hibi Liquid Hand Rub+ and Hibi Gel Hand Rub+ and the membrane filtration 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 (VRSA) and -intermediate (VISA) MRSA strains (n = 2), vancomycin-resistant *Enterococcus* spp (VRE) strains (n = 3), metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa* (n = 2), a multidrug, carbapenem-resistant (MDR) *Acinetobacter baumannii* strain (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.

P1149 Study of effectiveness and antimicrobial activity of an alcohol-free, non-rinse antiseptic developed for skin disinfection in emergency situations

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Objectives: After an emergency, it can be difficult to find running water. However it is still important to clean your hands to avoid illness. The aim of this study was to investigate effectiveness and biocidal activity of an alcohol-free, nonrinse antiseptic combination containing as active substances polyhexamethyleneguanidine phosphate (0.8%) and quaternary ammonium compounds (0.35%) and to show its advantages in respect to alcohol-based formulations for hygienic skin disinfection in emergency situations.
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*, *Ps. aeruginosa* and virulicidal against Hepatitis B virus in 15 seconds, fungicidal against *C. albicans* and tuberculocidal against *Mycobacterium* B5 in 1 minute and virulicidal against Polyovirus 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 non-flammable, 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 anavailable.

Diagnosis of bacterial infections

P1150 Nasal and throat swab cultures for the assessment of *S. aureus* colonisation and carriage in the Tromsø Staph and Skin Study. A validation study including standard screening methods and molecular typing

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Objective: More detailed epidemiological evidence about persistent *Staphylococcus aureus* carriage and its determinants are needed. In this perspective, adequate differentiation between persistent and non-or-intermittent carriage is a prerequisite. Isolation of *S. aureus* in repeated cultures is generally used to define persistent carriage. Our aim was to assess the intra-method reliability of nasal and throat swab cultures performed by two technicians, and to assess the inter-method reliability of two methods which both measure *S. aureus* carriage but with different time intervals between repeated nasal and throat swab cultures.
Methods: A validation study was performed autumn 2007 among 58 women and 50 men in The Tromsø Staph and Skin Study (TSSS), a large population-based study including 3,996 participants. Mean age was 60 years. A first set of repeated nasal and throat swab cultures was performed according to the TSSS protocol (culture 1 & 2; time interval between cultures; mean=14.3 days; standard deviation, SD=7.3 days), and a second set of repeated cultures was performed with one-week interval according to 'the culture rule' developed by Nouwen et al. (culture 3 & 4). Culture 2 and 3 were taken on the same day by two technicians about 60 minutes apart. All specimens were cultured within 24 hours on chromID *S. aureus* agar plates. Among 25 participants, *S. aureus* was identified in all 4 nasal cultures. All 100 *S. aureus* isolates from these chronic nasal carriers were spa typed according to Ridom StaphType 1.5 software.
Results: The inter-rater reliability was excellent for nasal culture (culture 1 vs 2; simple $\kappa = 0.94$; 95% confidence interval, 95%CI=0.87–1.00) and good for throat culture (simple $\kappa = 0.44$; 95%CI=0.21–0.67). The inter-method reliability was excellent for nasal carriage (culture 1 & 2 vs 3 & 4; weighted $\kappa = 0.85$; 95%CI=0.77–0.92) and good for throat carriage (weighted $\kappa = 0.44$; 95%CI=0.27–0.62). Among the chronic nasal carriers, all but one individual had *S. aureus* isolates that could be typed. In 18 carriers all 4 isolates were of identical spa types, 3 carriers had genetically closely related isolates, while 3 carriers had two non-related *S. aureus* types.
Conclusion: We confirm by standard screening methods and advanced molecular methods that nasal culture is a highly reliable and robust test for *S. aureus* colonisation and carriage. Throat culture has low reliability, and its role in research and clinics is highly questionable.

P1151 Improvement of a latex agglutination assay for specific detection of Panton-Valentine leukocidin

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Objectives: Panton-Valentine Leukocidin (PVL) consists of two components, LukS-PV and LukF-PV, which are secreted by some *Staphylococcus aureus* strains. Clinical isolates harbouring PVL gene are considered to be associated with severe soft-tissue infections and fatal necrotic pneumonia, and such cases are increasingly reported in communities.
Currently, PCR is widely used for PVL gene detection, but is often inconvenient and not easy to perform. In recent years we have reported the development of a simple Reverse Passive Latex Agglutination (RPLA) method, by using the anti-PVL specific polyclonal antibodies. Its performance correlated extremely well to PVL gene presence. However, it was difficult to prepare these specific antibodies and therefore we made improvements resulting in a new RPLA reagent with monoclonal antibodies, which are comparatively easier to produce.

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 37ºC 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.

P1152 Could the *Streptococcus pneumoniae* immunochromatographic test applied to nasopharyngeal aspirate be useful for diagnosing pneumococcal pneumonia?

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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 *Chlamydophila 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.

P1153 Rapid diagnosis by antigen detection for group B *Streptococcus* carriage in pregnant women

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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 strepto 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.

P1154 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*

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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 35ºC and 10 µl 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

at 35ºC. Identification of GBS were based on colony morphology, Gram staining, catalase reaction and latex agglutination tests (DiaMondiaL Strep Kit®) for all probable GBS grown on the CIDSB and CSBA. All discordant results were confirmed by latex tests and Api® 20 strep and statistical significance ($p < 0.05$) for the differences in recovery rates was obtained by using the McNemar's test.
Results: GBS were recovered from 98 specimens. The yields for GRA, CIDSA and SBA after 24(48)h of incubation were 91(91); 92(97) and 80(87) and overall sensitivities(specificities) of 92.9(100); 99.0(94.5) and 88.8(100)% were observed. No significant difference was observed between the GRA and either CIDSA or SBA but CIDSA performed better than SBA ($p < 0.01$). The positive predictive values for GRA and SBA were 100% and 95.8(84.3)% for CIDSA after 24(48 h) incubation. The prevalence of GBS and NHGBS in the study population was 23% and 1% respectively.
Conclusion: The better yield of the Granada® medium compared to the Strepto B® agar could be due to a better stability of the orange pigment. The ChromID® could be a good alternative since it would detect non haemolytic group B streptococci but it is more labour intensive as colonies are smaller, not well isolated and all typical colonies of GBS should be confirmed by latex tests.

P1155 *Legionella pneumophila* endocarditis: a diagnostic challenge

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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 16SrDNA primers followed by sequencing and BLAST analysis identified *L. pneumophila* both 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 claritromycin 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.

P1156 Evaluation of the Oxoid Xpect™ *Legionella* test kit for detection of *Legionella pneumophila* serogroup 1 antigen in urine

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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 −70ºC 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. Sensitivity 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 Oxoid Xpect compared to BinaxNow was found.
Conclusion: The study provided data showing that the Oxoid Xpect™ 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.

P1157 *Mycoplasma hominis*: an incidental but significant finding by routine bacteriological culture

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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 ºC in 5% CO2. The finding of translucent, pinpoint colonies after 96–120 h of incubation raises the suspicion of *M. hominis*; support for the 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 genitourinary procedures.

Four female patients with an incidental finding of *M. hominis* by routine bacteriological culture.

Patient	Age	Primary procedure	*M. hominis* isolated from	Empirical therapy	Susceptibility and therapy
1	23	Acute caesarean section (PROM and chorioamnionitis)	Cervix Placenta	Ampicillin & metronidazole (6 days)	Clindamycin: S Tetracycline: S
2	31	Elective caesarean section	Intraperitoneal abscess Surgical site infection	Cefuroxime & metronidazole (8 days)	MIC moxifloxacin: 0.047 μg/mL MIC ciprofloxacin: 0.094 μg/mL Tetracycline: S Clindamycin: R
3	35	Vaginal delivery (complicated by uterine perforation)	Peritoneal fluid	Ampicillin (8 d) → Cefuroxime (13 d) & metronidazole (21 d)	Tetracycline: S
4	56	Vaginal hysterectomy (metrorrhagia)	Vaginal abscess Renal abscess	Ampicillin & gentamicin & metronidazole (10 days)	MIC moxifloxacin: 0.023 μg/mL MIC ciprofloxacin: 0.094 μg/mL Tetracycline: S Clindamycin: S

P1158 The comparation of the fresh and lyophilised horse serum fertility: the effect on *Mycoplasma hominis* growth

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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 identification 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 5±3ºC (L2) and b) 6 months later, using the lyophilised horse sera stored for 9 months at 5±3ºC (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 statisticaly significant difference between them (p 0.724552581, χ-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 5±3ºC. 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.

P1159 Biotyping of *Brucella melitensis* using Fourier transform infrared spectroscopy

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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 Cygmus 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, 890, 944, 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.

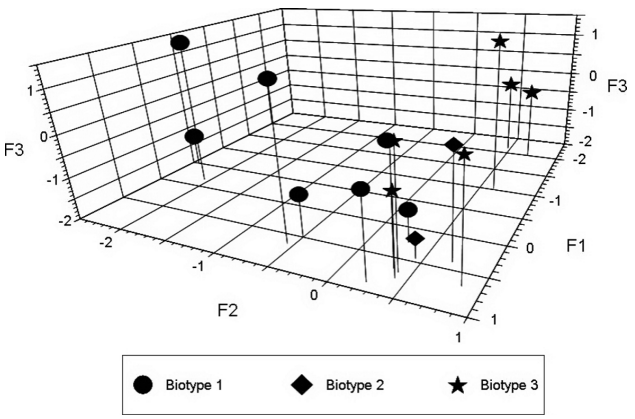

Figure 1. Distribution of *Brucella melitensis* biotypes.

Conclusion: FTIR may be useful in the characterisation of biotypes of *B. melitensis*.

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P1160 Performance of the BioPlex 2200 Syphilis IgM automated bead immunoassay system

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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 IgM 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

BioPlex 2200	SYPHILICHECK IgM Capture			
Syphilis IgM	Positive	Equivocal	Negative	Total
Positive	122	17	22	161
Equivocal	4	2	0	6
Negative	53	79	446	578
Total	179	98	468	745

Table II: BioPlex 2200 Syphilis IgM vs Immunoblot

BioPlex 2200	Immunoblot			
Syphilis IgM	Positive	Equivocal	Negative	Total
Positive	108	37	16	161
Equivocal	2	2	2	6
Negative	2	17	114	133
Total	112	56	132	300

Table III: SYPHILICHECK IgM Capture vs Immunoblot

SYPHILICHECK	Immunoblot			
IgM Capture	Positive	Equivocal	Negative	Total
Positive	103	24	52	179
Equivocal	4	19	75	98
Negative	5	13	5	23
Total	112	56	132	300

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

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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. Total incubation time is 2 hours. recomBlot Treponema IgG/IgM is a western blot kit that separate detection of IgG and IgM antibodies using pathogen specific Treponema antigens Tp47, Tp17, and Tp15 as well as TmpA.

Results: An overall 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 aginst 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.

P1162 Development of recombinant *Helicobacter pylori* CagA protein fragments for antibody production and characterisation

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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.

Methods: To develop *Escherichia coli* strains, producing recombinant CagA fragments (rCagA), 4 DNA overlapping fragments of cagA-gene were amplified and cloned into expression vectors. The N-terminally His6-tagged recombinant proteins were expressed in *E. coli* upon IPTG induction and purified by means of affinity chromatography on Ni-NTA. The pure protein samples were used for F1(SJLxBALB/c) mice immunisation. The splenocytes of immunised animals were hybridised with myeloma cells to produce hybridoma lines. Hybriodoma screening and MAbs characterisation were performed by means of ELISA and Western-blot, with rCagA and *H. pylori* strains protein extracts being used as antigens.
Results: Four *E. coli* strains expressing recombinant CagA fragments of 75, 65, 44, 39 kDa with N-terminal His6-tag were constructed. More than 20 hybridoma cell lines were obtained and proved to produce antibodies against CagA. Four MAbs had the highest affinity and to recognized different isolated linear epitopes on rCagA, with one epitope being localised inside the variable region of CagA. Two of four MAbs were shown to bind full-length CagA protein (>120 kDa) in samples from *H. pylori* strains. The efficacy of discrimination between CagA-positive and -negative *H. pylori* strains by means of these MAbs turned out to be comparable with that of commonly used PCR-diagnostics. A sandwich-ELISA system was constructed for quantitative detection of CagA protein in biological samples and estimated the sensitivity of this assay.
Conclusion: The obtained 4 recombinant CagA fragments may be used further as standardised antigen reagents for epidemiological screening of *H. pylori* infection and for vaccination results estimation. MAbs against CagA can be used for detection of infections with the most virulent CagA-expressing *Helicobacter* and for research purposes as well.

P1163 Evaluation of a monoclonal-based antigen in stool enzyme immunoassay for diagnosis of *Helicobacter pylori* infection in Spanish children

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Objectives: *Helicobacter pylori* infection can be diagnosed by invasive and no invasive methods. Invasive tests, including culture, histology and rapid urease, it 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 pacients with gastric 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 identificacion. 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 immunosorbent 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 assenssing 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

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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 expansive 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 m to 14.9 y; 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.

	Rapid Hp StAR	H. Pylori Antigen Test	Medcard Pylori	Pylori Strip	H. Pylori Stool Card	ImmunoCard STAT
Sensitivity	76.9%	76.9%	84.6%	61.5%	76.9%	38.5%
Specificity	92.3%	84.6%	88.5%	100.0%	84.6%	100.0%
PPV	83.3%	71.4%	78.6%	100.0%	71.4%	100.0%
NPV	88.9%	88.0%	92.0%	83.9%	88.0%	76.5%

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

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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 diarhhoea or fever) living in Bandung, West Java Indonesia. All faeces samples were tested by a rapid test (Coris BioConcept, Gembloux 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%, 98.2%, 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

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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 of110(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 andPPI.Urine,stool and serum specimens from these patients were examined and evaluated pre-treatment,at6thweek and at6thmonth after eradication therapy by URINELISA (Otsuka Pharmacautical, 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%) and48 (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 *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 week, 19(37.3%) were diagnosed as *H. pylori* positive by UBT, 32 (62.7%) patients were succesfully treated. 25 (49.1%) and 37 (72.6%) were positive by OD 1 and OD 0.397 URINELISA.The sensitivity, specificity, PPVand NPVwere 52.6%, 79.0%; 53.1%, 31.3%; 40.0%, 40.6% and 65.4%, 37.3% respectively (K 0.054) (K 0.086). 22 (43.1%) were positive by the Rapid Hp StAR test. The sensitivity, specificity, PPVand NPV were 79.0%, 78.1%, 68.2% and 86.2%, respectively(K 0.553). 43 (84.3%) were positive by *H. pylori* IgA/IgG ELISA. The sensitivity, specificity, PPV and NPV were 89.5%, 18.8%; 39.5%, 75.0%, respectively (K 0.065). In 6th month, 15 (29.4%) and 24 (47.1%) were positive by OD 1 and OD 0.397 URINELISA, 20 (39.2%) were positive by the Rapid Hp StAR test and 34 (66.7%) were positive by *H. pylori* IgA/IgG ELISA.We also compared all results in pre-treatment, 6thweek and 6thmonth after eradication therapy by Cochran analysis.
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 6thmonth 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 6thmonth besides pre-treatment.

P1167 A two step algorithm, toxin immunoassay and stool culture, for the diagnosis of *C. difficile* infection where TcdA-TcdB+ variant strains are prevalent

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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 immunoassay (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 immunoassay (ELFA, VIDAS CDAB, Bio-Merieux sa, Marcy-l'Etoile, 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.
Conclusion: 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 immunoassays for diagnosis of *Clostridium difficile* toxin A and toxin B

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Objective: Enzyme immunoassay (EIA) 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 immunoassay (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 gene 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.

P1169 Evaluation of two algorithms using GDH and toxin A&B enzyme immunoassays for rapid diagnosis of *Clostridium difficile* infection

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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 toxin 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% 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.

P1170 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

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Objective: The aim of this study was to evaluate the performance of the chromIDTM 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 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.

P1171 Enhancement of motility ability of viable thermophilic *Campylobacter* in viscous condition

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Objectives: 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 18 h. 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 $10–10^4$. 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 37ºC and 42ºC for 24 h. Drop plate method was used to determine numbers of viable *Campylobacter* at 6, 12, 18, and 24 h.
Results: *Campylobacter* moved through the soft-agar-coated filter at both 37ºC and 42ºC. After 6 h, 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 42ºC were higher than those at 37ºC. Following the inoculation of *Campylobacter* (10^4 cfu/g), the numbers of cells at 42ºC was 10^4 cfu/ml and 10 cfu/ml, at 37ºC after 6 h inoculation. The highest concentration of the cells reached 10^9 cfu/ml at 42ºC and 10^7 cfu/ml at 37ºC, after 24 h inoculation. When the inoculation was 10 cfu/g, the numbers of cells at 42ºC was 10^3 cfu/ml and 10^2 cfu/ml, at 37ºC after 6 h inoculation. The numbers of cells at 42ºC was 10^6 cfu/ml and 10^4 cfu/ml, at 37ºC 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.

P1172 Monitoring the evolution of brucellosis by using four common serological tests

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Objectives: 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 the

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, Immunocapture-agglutination test (Brucella capt).
Results: The serological titers of 3 patients were not correlated with active disease (STA $\leqslant$ 80U, RB negative, Elisa IgM < 15 U/ml, IgG 20–50 U/ml, Brucellacapt $\leqslant$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 Brucellacapt test positive (ranged from 640 up to10240 U), while in 7/14 and in 10/14 patients STA test and RB test were respectively positive.
Conclusion: 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. Brucellacapt 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.

P1173 Relationship between Guillain-Barré syndrome and *Coxiella burnettii* infection

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Objectives: Several bacterial and viral agents have been implicated in the pathogenesis of the Guillain-Barré syndrome, an acquired inmunomediated disorder. In the *Coxiella burnettii* 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 patien 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 bacterias.
Results: The first patient was diagnosed of myelitis, and the second of Miller-Fisher syndrome. In both of them, serological procedures showed *Coxiella burnettii* 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 burnettii* IgG antibodies of 1:32 and high levels of proteins.
Fourteen days doxyciclin treatment (200 mg. daily) induced a rapid decreasing of antibodies levels, with a rapid recovery of the most of neurological symptoms.
Conclusion: *Coxiella burnettii* should therefore be added to the list of microorganisms capable of inducing the Guillaim-Barré syndrome.
Serological testing should be performed in cases of meningoencephalitis, lynphocytic meningitis, and peripheral neuropathy including Guillain-Barré syndrome and myelitis.

P1174 Clinical significance of (1.3)-b-d-glucan detection in *Pneumocystis jirovecii* pneumonia

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Objectives: To evaluate the clinical usefulness of Fungitell test (Associates of Cape Cod, Inc., Cape Cod, MA) – an immunological assay detecting (1.3)-b-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/mmc; full-dose corticosteroid treatment for >8 weeks; haematologic malignancy 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

P1175 *S. aureus* bloodstream infection and time-to-positivity

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Objective: The aim to this study is to asses 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 13.18 h (range from 51 minutes to 30.58 hours). The median time of MSSA (12.78 h) was shorter than MRSA (15.02 h) although this difference was not significant (P = 0.08). The TTP was significantly shorter from patients with endocarditis (6.62 h) compared with the rest of sources (15.2 h) (P = 0.0001). However, in patients with catheter related infection 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 (<12 h), this group is associated with endocarditis (median time 5.2 h), catheter related (8.94 h), respiratory (8.11 h) and urinary (8 h). The overall mortality rate was 20.6%: MRSA

19.3% and MSSA 21.1%. The sources with higher mortality were gastrointestinal tract 50%, respiratory 48.6%, other bacteraemias named group 26%, primary 20.8% and catheter related 12.7%.
Conclusions: Patients in the group of MRSA bacteraemia are older than patients in MSSA group. Our data support the relationship of TTP with endocarditis and catheter related infection. Growth of *S. aureus* within 12 hours after the initiation of incubation may identify patients with a high likelihood of endocarditis, catheter infection and possible complications.

P1176 Evaluation of an agglutination method (Dryspot Pneumo®, Oxoid) for the direct detection of *S. pneumoniae* in blood cultures

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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. bovis*, 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.

P1177 Comparison of VersaTREK 528 and BACTEC 9240 continuous monitoring blood culturing systems for the detection of clinical isolates in a seeded study

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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 PedsPlus media were compared to VT standard aerobic and anaerobic media (80 and 40 mL bottles). Bottles were supplemented with 100 or 500 uL of human blood and inoculated with either 1×10^1 or 1×10^4 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.

P1178 Time to positivity in monomicrobial and polimicrobial fungal blood cultures

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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 (Bact/Alert, 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 polimicrobial 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 positve 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

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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 polyanethole-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 Aerobic/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 septicaemia 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 Plus 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 bottle, the Peds Plus/F bottle was not 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/Anaerobic blood culture set would probably increase the detection rate of pathogenic bacteria by 1% of the total number of blood culture sets taken. The cost of a third vial will be around 400€ a year and then more expenses for identification etc.

Table 1. Comparative times to detection in Plus Aerobic/F and Peds Plus/F blood culture bottles when both bottles were positive

Microorganism(s)	No. of isolates	Time to detection (h), median (range)		*P* value
		Plus Aerobic/F	Peds Plus/F	
Staphylococcus aureus	50	16.7 (8.9–119)	16.6 (7.9–114.3)	NS[a]
Coagulase-negative staphylococci[b]	27	25.5 (9.4–54.6)	21 (12.3–58.3)	0.001
Enterococcus spp.[c]	26	15.6 (8.2–40.2)	14.7 (7.5–115)	NS
Streptococcus spp.[d]	44	11.9 (3.2–61)	11.5 (4.7–45.6)	NS
Gram-positive bacilli[e]	2	28.7 (23.1–34.2)	37.3 (32.3–42.2)	NS
Escherichia coli	71	14.1 (1.8–66.8)	12.6 (4.3–123.8)	NS
Klebsiella spp.[f]	20	13.8 (4.9–50)	13.1 (3.7–66.5)	NS
Other Enterobacteriaceae[g]	17	15.6 (4–86)	13.7 (4.3–130)	0.006
Pseudomonas aeruginosa	4	16.2 (13.1–24)	15 (12.1–24)	NS
Other Gram-negative bacteria[h]	7	19 (12.5–38.8)	19 (12.5–40.8)	NS
Candida spp.[i]	11	49 (21.1–93.5)	67 (22.6–138.6)	0.002
Total	279	15.6 (1.8–119)	14.6 (3.7–138.6)	<0.001

[a] NS, not significant ($P > 0.05$).
[b] Includes 15 *Staphylococcus epidermidis*, 6 *Staphylococcus hominis*, 1 *Staphylococcus capitis*, 2 *Staphylococcus lugdunensis*, 3 *Staphylococcus warneri* and 4 coagulase-negative staphylococci.
[c] Includes 18 *Enterococcus faecalis*, 6 *Enterococcus faecium* and 2 *Enterococcus casseliflavus*.
[d] Includes 2 Group-A beta-haemolytic *Streptococcus* (HS), 2 Group-C HS, 3 Group-G HS, 25 *S. pneumoniae*, 2 non-HS, 4 *S. gordonii*, 2 *S. anginosus*, 1 *S. constellatus*, 1 *S. dys. equisimilis*, 1 *S. sanguinis* and 1 *S. gallolyticus*.
[e] Includes 1 *Corynebacterium* spp. and 1 *Corynebacterium* group G.
[f] Includes 14 *Klebsiella pneumoniae*, 5 *Klebsiella oxytoca* and 1 *Klebsiella ornithinolytica*.
[g] Includes 4 *Enterobacter cloacae*, 1 Enterobacteriaceae, 4 *Proteus mirabilis*, 1 *Salmonella enteritidis*, 1 *Salmonella typhimurium*, 1 *Salmonella dublin*, 1 *Salmonella wirschow*, 3 *Serratia marcescens* and 1 *Citrobacter braakii*.
[h] Includes 1 *Capnocytophaga* spp., 2 *Neisseria meningitidis* group B, 1 *Haemophilus parainfluenzae*, 1 *caps. H. influenzae*, 1 *Morganella morganii* and 1 *Pasteurella multocida*.
[i] Includes 8 *Candida albicans*, 1 *Candida lusitaniae* and 1 *Candida krusei*.

P1180 The clinical impact of pre-incubation of blood cultures at 37ºC

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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 37ºC in the laboratory. The impact of pre-incubation of blood cultures at 37ºC (i.e. holding blood cultures at 37ºC 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 37ºC 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 37ºC 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 37ºC resulted in a significant reduction of the turnaround time. Early positive blood culture results contributed importantly to appropriate antimicrobial management.

	March-Sept 2006 Pre-incubation 37ºC				March-Sept 2005 Room temperature			
ED visits with BC obtained	969				885			
BC bottles collected	3222 (3.33/visit)				3012 (3.40/visit)			
BC bottles stored at 37ºC/room temp.	2060/1162				0/3012			
Transport time per ED visit								
Unknown (no collection time)	192 (20%)				286 (32%)			
⩽8 hours	211 (27%)				161 (27%)			
9–16 hours	269 (35%)				189 (32%)			
16–24 hours	264 (34%)				152 (25%)			
>24 hours	33 (4%)				97 (16%)			
ED visits with positive BC	103 (11%)				106 (12%)			
Mean turnaround time (h)	24				36			
Day of consultation								
Unknown (no collection time)	21 (20%)				36 (34%)			
	D0	D1	D2	>D2	D0	D1	D2	>D2
⩽8 hours	4	18	3	2	1	15	2	1
9–16 hours	6	21	3	2		11	13	4
16–24 hours		16	3	2		3	16	2
>24 hours		2				1	1	
Total	12%	70%	11%	7%	1%	43%	46%	10%
Impact Gram stain BC on antimicrobial therapy								
Initiation of appropriate therapy	21%							
Streamlining of therapy	10%							

ED = Emergency Department, BC = Blood Culture, Transport time = time between BC collection and incubation in the laboratory. Turnaround time = the time between BC collection and Gram stain results of a positive BC. Day of consultation = the day BC turns positive, D0 = the day BC was collected. D1 = 1 day after BC collection. D2 = 2 days after BC collection. >D2 = more than 2 days after BC collection.

P1181 Evaluation of the new BACTEC FX instrument and a new modified BACTEC Plus Aerobic medium

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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 the 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.

P1182 Using digital tinctorial properties to rapidly distinguish *Pseudomonas aeruginosa* from other Gram-negative bacteria in bacteraemic patients

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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* ($p < 0.001$). 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

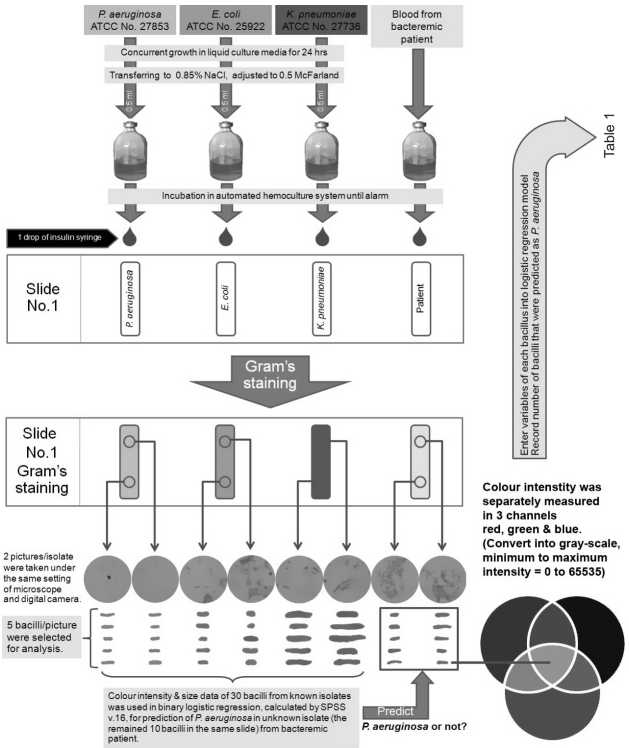

Table 1 *P. aeruginosa* predicted by logistic regression model

Slide No.	Bacteria	Predicted *P. aeruginosa* number	% correct
1	*P. aeruginosa*	10/10	100
2		8/10	80
3		3/10	30
4		5/10	50
5		9/10	90
6		5/10	50
7	*E. coli*	0/10	100
8		0/10	100
9		0/10	100
10		1/10	90
11		0/10	100
12		0/10	100
13		2/10	80
14	*E.coli & K. pneumoniae*	0/10	100
15	*K. pneumoniae*	0/10	100
16		0/10	100
17		0/10	100
18		0/10	100
19		0/10	100
20	*A. baumannii*	3/10	70
21		0/10	100
22		0/10	100
23		0/10	100
24		0/10	100
25	*B. cepacia*	7/10	30
26	*E. cloacae*	0/10	100
27	*Salmonella gr.B*	0/10	100
28	*S. marcescens*	0/10	100
29	*S. maltophilia*	1/10	90
30	*Moraxella sp.*	8/10	20

For example, slide No.1, all bacilli in picture of unknown isolate from patient were correctly predicted as *P. aeruginosa* (10/10 bacilli)

haemoculture were correctly identified. Incorrect predictions were seen in pseudomonas-related species (*B. cepacia*, *S. maltophilia*), *Moraxella* sp., *E. coli* and *A. baumannii*. *Moraxella* sp. and *A. baumannii*, however, can be distinguished by their diplococcal morphology.
Conclusion: Our study demonstrates a potential utility of digital tinctorial measurement as a diagnostic aid to guide appropriate empiric antimicrobials. Further analysis on more bacterial genera and from more specimens are needed.

Bloodstream infections

P1183 Approaching zero rates bloodstream infections in a tertiary care neonatal intensive care unit: a multifaceted approach

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Objective: To identify the trend of healthcare associated bloodstream infection (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 CLA-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 CLA-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

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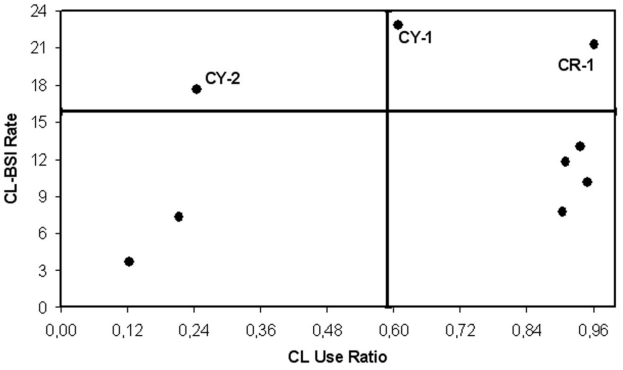
Objective: Surveillance of intensive care unit (ICU) acquired infections has become an integral part of infection control programmes in several countries, and outcome and process indicators are increasingly used in benchmarking the quality of medical care. The objective of this study was to implement 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)] was utilised as a process indicator and the CL-BSI rate [(number of primary CL-BSIs)/(number of CL-days)$\times$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.
Conclusion: 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

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Objectives: Catheter-related bacteraemia 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 term tunneled central venous catheters.
Methods: From July 2003 to January 2006, we conducted a prospective study to detect CC, and establish a pre-emptive 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 theirs relationship to CC and CRBI in haemodialysis patients. These characteristics were analyzed by Kaplan-Meier survival curves. Statistical differences between survival curves were determinated 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=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 CC (HR=3.72, 95% CI, 0.99–13.87, P = 0.050) and duration of catheter use (HR=3.57, 95% CI, 1.52–8.36, P = 0.003) were identified as risk factors for CRBI. *S. aureus* nasal carriage was risk factor in CRBI episodes caused by *S. aureus* ('$\chi 2$' = 5.585, 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* nd methicillin-sensitive *Staphylococcus aureus* bloodstream infection in a Leicester cohort of patients; Panton-Valentine leukocidin gene prevalence in the same cohort

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Objectives: To compare predictors for outcomes in patients with MRSA and MSSA bacteraemia; 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, speciality, 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 bacteraemia, (58 (48%) MRSA, 62 (52%) MSSA). 30 day mortality was 31/62 (50%) in the MRSA group, 13/58 (22%) in the MSSA group: $\chi^2 = 9.82$, p = 0.002. Univariate analysis identified differences in mean age (t = 4.71, p = 0.000, CI: 8.9–21.8), higher mean sepsis score (t = 5.68, p = 0.000, CI: 7.46–15.2), previous colonisation with MRSA ($\chi^2 = 9.73$, p = 0.008), quinolone resistance ($\chi^2 = 10.23$, p = 0.05) and underlying co-morbidities (t = 2.1, p = 0.035, CI: 0.03–0.8) as statistically significantly associated with 30 day mortality. The source of staphylococcal bloodstream infection, gender, speciality and length of stay prior to positivity were not identified as associations. The significant associations were analysed by backward step logistic regression: meticillin resistance, p = 0.042, quinolone resistance, p = 0.014 and higher mean sepsis score, p = 0.000 were independent predictors of mortality. 107 of the samples were tested by PCR for the PVL gene: 2 positive = 0.02%, 1 MSSA and 1 MRSA, both associated with skin/soft tissue infection and both patients died.
Conclusions: Meticillin resistance, higher sepsis score and quinolone resistance were independent predictors of mortality in staphylococcal bloodstream infections. The PVL gene was present in only 0.02% of the population but the sample size was too small to attach any statistical significance.

P1187 Risk factors for methicillin resistance and factors associated with in-hospital mortality during *Staphylococcus aureus* blood stream infection: an observational study

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Objective: *Staphylococcus aureus* is one of the most common aetiologies 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. The study was approved by Pontchaillou institutional review board. 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 70/36, 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 intravenous 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 $\geqslant$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 $\geqslant$ 3 remained predictive of mortality on multivariate analysis (OR 2.07 [1.80–2.85]; P = 0.001).
Conclusion: 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 bacteraemia in critically ill patients

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Objective: To analyze the risk factors involved in the development of carbapenem resistant Gram-negative bacteraemia (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 60% 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 higher in patients with carbapenem resistant GNB, compared to those 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 $\leqslant$ 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

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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, 21.33%), *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 hospital stay until Gram-negative bacteraemia was 19±17 (range 3–82) 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 APACHE II scores, intensive care unit stay, mechanical ventilation support, existence of arterial line and central venous catheter, receiving TPN, hypotension, hypothermia, coma, elevated levels of urea, and in vitro resistance to amikacin and carbapenems. Antibiotic treatment was inappropriate in 33 (13%) patients. Mortality rate was 72.7% in patients receiving inappropriate antibiotic treatment and 32.3% in patients receiving appropriate antibiotic treatment ($p < 0.001$) at day 14. Independent risk factors for mortality were having an APACHE II score over 20 (OR: 3.18, CI: 1.9–5.2, $p = 0.001$), receiving ICU care (OR: 5.43, CI: 1.7–17.4, $p = 0.004$), inappropriate antibiotic treatment (OR: 2.38, CI: 1.2–4.7, $p = 0.013$), receiving TPN (OR: 3.96, CI: 1.8–8.9, $p = 0.001$), coma (OR: 3.7, CI: 1.4–9.6, $p = 0.007$) at day 14 in logistic regression model.
Conclusion: 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

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Objective: The aim of our study was to compare characteristics and outcome in medical patients with nosocomial and community-acquired bloodstream infections (BSI).
Patients-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±31.0 vs. 72.8±33.4, respectively, $p = 0.049$; WBC: 16.1±8.3 vs. 13.0±5.3×10^3 cells/mm^3, 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

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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 (1.03/1000 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=.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=.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

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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 ceftazidime, 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 ceftazidime 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.

P1193 Enterococcal bacteraemia in a Swiss tertiary-care hospital: epidemiologic risk factors and outcome

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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, CI95 1.2–18.6) and complication rates ($p = 0.004$; OR 3.86, CI95 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, CI95 1.1–19.4) and intraabdominal infections (22.2% versus 5.8%, $p = 0.024$, OR 4.63, CI951.1–19.4) 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

Risk factor	Odds ratio	p-value	95% confidence interval
ICU stay	2.66	0.097	0.84–8.46
Haematologic malignancy	4.50	0.062	0.93–21.81
Organ transplant	6.06	0.016	1.39–26.39
Dialysis	2.08	0.276	0.56–7.75
Prior fluoroquinolone treatment	8.13	0.008	1.75–37.77
Prior piperacillin-tazobactam treatment	1.30	0.702	0.35–4.78
Prior glycopeptide treatment	1.82	0.424	0.42–7.89

P1194 Growth inhibition of micro-organisms involved in catheter-related infections by an antimicrobial transparent IV dressing containing chlorhexidine gluconate

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Introduction: Infections associated with intravascular devices represent 10 to 20% of all nosocomial infections and are mostly caused by microorganisms belonging to 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 10^8 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 35ºC, the diameter of the zone of inhibition was measured.

Table 1. Strains of tested microorganisms and zones of growth inhibition

Organisms			Millimetres (mm) of zone beyond dressing
Staphylococcus epidermidis	MRSE	ATCC 51625	8.7
	MRSE	nasal isolate #492	8.6
Staphylococcus aureus	MRSA/GRSA	ATCC 33592	7.3
	MRSA	nasal isolate #849	5.7
Pseudomonas aeruginosa		ATCC 27853	4.5
Enterococcus faecium	VRE	ATCC 700221	7.1
	MDR	ATCC 51559	7.9
Enterococcus faecalis	VRE	wound isolate #23	4.3
Klebsiella pneumoniae		ATCC 13883	4.4
		ATCC 23357	3.6
Acinetobacter baumannii		wound isolate #12-4	4.6
Enterobacter cloacae		ATCC 35549	4.3
Candida albicans		ATCC 10231	7.3
		ATCC 58716	4.9

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 methicillin 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

P1195 Heterogeneous population structure by MLST of extended spectrum β-lactamase producing *Klebsiella pneumoniae* isolates

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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 at 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 pulsotypes and belonged to the phylogroup KpI. 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-Courlin 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.

ST	ESBL (no. isolates)	Year	Sample
ST13	CTX-M-15	2005	Urine
ST14	CTX-M-10	1990	BAS
	TEM-4	1997	Blood
	SHV-12	2004	Urine
	TEM-4 (3)	1995–1999	Sputum, BAS (2)
ST15	CTX-M-15	2002	Urine
ST16	CTX-M-15 (2)	2004	Urine
ST20	CTX-M-10 (2)	2000–2001	Sputum, Urine
ST23	CTX-M-10	2000	Urine
ST34	CTX-M-10	2003	Respiratory
ST37	SHV-12	2003	Catheter
ST39	TEM-4	2001	BAS
	SHV-12	2003	Urine
ST101	SHV-12	2005	Blood
ST111	CTX-M-10	1993	Blood

P1196 Ciprofloxacin-resistant and CTX-M-15-producing *Escherichia coli* from extra-intestinal infections in Italy

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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 topoisomerase genes: Ser83Leu and Asp87Asn in gyrA; Ser80Ile 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 B-lactamase

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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 b-lactamases (ESBLs) were collected between 2001 and 2007 in a Hospital Santa Maria, in Lisboa. 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 73.3% (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, N and Y plasmid 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 profiles 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

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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-9, 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

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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′ flanking 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.

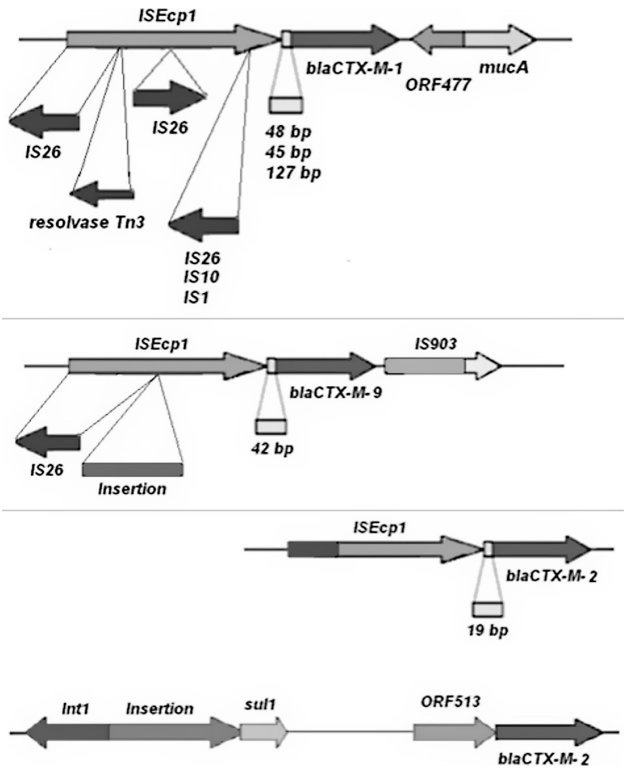

Figure 1. Genetic environments of blaCTX-M genes (blaCTX-M-1, -9, and -2 subgroups) – location of rearrangements in different strains.

P1200 Characterisation of IncHI2 plasmids carrying extended-spectrum β-lactamase genes

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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 (DQ517526), 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-mediated 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

compared. Most of these plasmids were associated to the international spread of ESBL and PMQR and carried the blaCTX-M-2, blaCTX-M-9, blaSHV-12, blaLAP-2 and qnrS genes. These plasmids are very difficult to analyze by standard methods, being >250 kb in size, thus the characterisation was performed by allelic variation analysis and Southern blot hybridisation performed on total DNA restricted with EcoRI or PstI. Allelic studies were performed on the smr0017 and smr0199 genes, showing sequence heterogeneity between R478 and pAPEC-O1-R. smr0017 belongs to the plasmid partitioning cluster and smr0199 encodes a bundle-forming pilus biogenesis protein. R478 and pAPEC-O1-R scaffolds were designated as sequence types (ST) ST1 and ST2, respectively.
Results: All IncHI2 plasmids carrying blaCTX-M-9 and blaSHV-12, despite their origin, source and country of isolation were ST1 and R478 derivatives, while those carrying blaCTX-M-2, blaLAP-2 and qnrS genes were ST2 and pAPEC-O1-R derivatives.
Conclusion: blaCTX-M-2 and blaCTX-M-9 genes demonstrated successful dissemination among different enterobacterial species from both animal and human sources in very distant geographical regions, through the epidemic spread of two major IncHI2 plasmid variants. This result is of concern since these successful, epidemic plasmids could promote in the next future and with the same efficiency, the worldwide spread of other resistance genes, suggesting the necessity of monitoring their diffusion in Enterobacteriaceae.

P1201 Dispersal and persistence of TEM-4 in Spain: a plasmid-clone paradigm

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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 (I-CeuI) 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 hybridisation), HpaI-RFLP, and screening for colicins (cbi, cma y cvaC). The presence of class 1 integrons, Tn402 (orf5, IS1326, IS1353, 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 most transconjugants showing resistance to gentamicin and tobramycin. blaTEM-4 was located in different plasmid RFLP-types (35–120 kb): i) pRYC106 (IncFII-80 kb, reppC15–1a, cbi +), identified in a D-EC epidemic clone (1989–1997); ii) pRYCE11 (80–90 kb, untypeable), an epidemic plasmid recovered from KP (n = 7 clones, 1995–2004) and B2-EC (n = 2 clones, 1991–1999); iii) pRYC107 (35 kb, untypeable), isolated in 3 EC clones (1991–1999); iv) pRYC108 (80 kb-IncI1, repR64 sequence), detected in 2 EC clones (2000–01); and v) 2 IncI1-like plasmids (50–120 kb). Tn3-like sequences were identified in all plasmid types. Class 1 integrons containing aadA1 cassette were occasionally detected and associated with the presence of orf5, IS1326 and/or IS6100, and presumptively linked to left (tnpR-tnpM) and right (merA, IRt) arms of mercurial Tn.
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.

P1202 Updated epidemiology of extended spectrum β-lactamase producing *Enterobacter* isolates in a university hospital in Madrid, Spain (2001–2007)

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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 conjugation 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, which contrast with previous situation, 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) were identified whereas ENA harboured TEM-24 and only in one case a TEM-4. Transference 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 ac.; 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 ac., 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, ENC 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 fluoroquinoles 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 qnrB9 element and blaTEM-116 ESBL-gene in *Citrobacter freundii* environmental strain

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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

were determined by the conventional macrodilution broth procedure, according to the CLSI guidelines.
Results: *Citrobacter freundii* AQ/1, selected for resistance to nalidixic acid, showed a large plasmid named plCHIGR 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(plCHIGR) and *E. coli* recipient cells. Both *C. freundii* AQ/1 and *E. coli* HB101(plCHIGR) were resistant to penicillin, 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 values for *C. freundii* AQ/1 and *E. coli* HB101(plCHIGR) of 0.25 and 0.5 mg/L, respectively.
Conclusion: Our finding suggests how qnr elements and blaESBLs genes are easily co-transferred among Enterobacteriaceae in different environments.

P1204 First report of TEM-52 β-lactamase in *Proteus mirabilis* strains from Croatia

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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 rifampicin. 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, cefoxitin, 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

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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-N020 card in the Vitek 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 (AB13100) 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 Aspertine (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

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Objective: To characterise the genetic determinant responsible for the ESBL phenotype in a *Providencia rettgeri* bacteraemia 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 ($\geqslant$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, VEB, 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 bloodculture 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 admittission for evaluation of pneumonia. He was briefly hospitalised a month early 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 aminoacid 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.

P1207 Variability of determinants conferring extended-spectrum β-lactamase resistance in German *Salmonella enterica* serovar Paratyphi B d-tartrate + (S. ser. Java) isolates

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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 ceftiofur $\geqslant$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 ceftiofur, 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 qnrA, B, 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), in which a class 2 integron is chromosomally located, has evolved to cephalosporin resistance by acquisition of plasmids containing different ESBL-encoding genes. Like in other middle European countries in Germany an increase in the number of 3rd generation cephalosporins and fluoroquinolone resistant in S. Paratyphi B dT+ isolates, specially originating from foods of avian origin, could be observed.

P1208 Prevalence of extended-spectrum β-lactamases in *Escherichia coli* obtained from faecal samples of captive ostrich in Portugal

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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 24 h at 37ºC; 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, cnf1, 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 sulphametoxazole resistance), as well as the aer and fimA virulence genes, being the three isolates of the B1 phylogroup. They showed resisstance to nalidixic-acid, ciprofloxacin, tetracycline, and streptomycin, in addition to β-lactams, and two of them also to chloramphenicol or trimethoprim-sulphametoxazole. Class 1 integrons were identified in the three ESBL-positive *E. coli* isolates with the following gene cassette arrangements 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

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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 plate counts on MacConkey agar. After overnight incubation, broths were platted onto MacConkey agar supplemented with 1 mg/l cefotaxime or ceftazidime. Presumptive *E. coli* colonies were identified by standards methods. ESBL production was determined by the double disk diffusion method and further characterisation was carried out by pI, PCR with specific primers for bla-genes and sequencing. Phylogenetic group was assigned by multiplex PCR.
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 Log_{10} 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 dehydrating 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.

P1210 Prevalence of antimicrobial resistance and antimicrobial resistance genes among *Escherichia coli* from healthy volunteers and patients with urinary tract infection

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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, cephalexin, 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-2 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.

Antimicrobial	Resistance (%) among *E. coli*		χ^2 p value
	commensal (n = 295)	urinary (n = 295)	
Amoxicillin	32.9	49.8	<0.001
Cephalexin	4.7	5.1	0.850
Ciprofloxacin	2.7	8.8	0.001
Co-amoxiclav	4.4	5.4	0.568
Ertapenem	2.4	0.3	0.075
Gentamicin	3.4	7.8	0.020
Meropenem	0.0	0.0	1.000
Nitrofurantoin	2.7	5.1	0.136
Tetracycline	18.0	32.5	<0.001
Trimethoprim	15.3	30.5	<0.001

P1211 Evaluation of the frequency antibiotic resistance genes by Identibac TubeArray in CTX-M ESBL *E. coli* of veterinary origin

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Objectives: Extended spectrum β lactamases are important in both human and veterinary medicine as they inactivate front line third generation cephalosporin antibiotics. The CTX-M ESBLs are carried and transferred on plasmids which may also contain other antibiotic resistance genes. The first veterinary isolation of CTX-M producing *E. coli* in the U.K. was from a large dairy farm in 2004 (Liebana et al., 2006). The objective of this study was to determine the frequency of other antibiotic resistance genes in CTX-M bearing *E. coli*.
Methods: A panel of 157 CTX-M ESBL *E. coli* of veterinary origin was analysed by Identibac ArrayTube for the detection of antimicrobial resistance genes in Gram-negative bacteria. The panel of *E. coli* included isolates from 54 different locations, and where appropriate, pulse field gel electrophoresis was used to select individual clones for analysis. The ArrayTube includes genes encoding resistance to aminoglycosides, trimethoprim, sulphonamides, tetracyclines, and β-lactams including the extended spectrum β-lactamases (Batchelor et al., 2008).
Results: Most of the CTX-M genes (68.2%) hybridised with the group1 probe. The most frequently occurring genes found in the CTX-M ESBL *E. coli* isolates were TEM-1 (65.6%), tetA (61.1%), strB (59.9%) and sul2 (59.2%). The frequency of other antibiotic resistance genes occurring with CTX-M group1, TEM-1, tetA, strB and sul2 are provided in table 1.
Conclusion: CTX-M ESBL *E. coli* frequently carried other antibiotic resistance genes, particularly those providing reduced susceptibility to other β-lactams, tetracycline, streptomycin and sulphonamides. These were easily and rapidly detected using the Identibac ArrayTube. Further studies with transconjugants or transformants will be used to investigate gene linkage and provide a better understanding of the epidemiology of different isolates.

Table 1. Frequency of other antibiotic resistance genes occurring with CTX-M group 1, TEM-1, *tetA*, *strB* and *sul2*

TEM-1	*tetA*	*strB*	*sul2*
strB (72.1%)	TEM-1 (70.8%)	TEM-1 (79.8%)	*strB* (77.4%)
CTX-M1* (70.2%)	CTX-M1 (69.8%)	*sul2* (76.6%)	TEM-1 (75.3%)
sul2 (67.3%)	*sul2* (66.7%)	CTX-M1 (65.9%)	CTX-M1 (68.8%)
tetA (65.4%)	*aadA1* (65.6%)	*sul1* (64.9%)	*tetA* (68.8%)
tetB (60.6%)	*intI1* (61.5%)	*tetA* (62.8%)	*sul1* (66.7%)
aadA1 (58.7%)	*strB* (61.5%)	*tetB* (61.7%)	*tetB* (64.5%)
sul1 (56.7%)	*sul1* (53.1%)	*aadA1* (58.5%)	*intI1* (62.4%)

*CTX-M1 probe hybridises with CTM-M group 1 genes including $bla_{\text{CTX-M1,3,10,12,15,22,23,28}}$ and FEC-1.
Intl1 indicates class 1 integrase.

P1212 Characterisation of CTX-M-positive isolates of Enterobacteriaceae using real-time PCR and melting curve analysis

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Objectives: 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 patiens 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-disk 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 family 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 types β-lactamases was approved.
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P1213 Identification of a chromosomal class A β-lactamase from *Serratia rubidaea*

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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 (AmpC-type of *S. marcescens*), class A (FONA of *S. fonticola*) or class B (Sfh-I 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 pI 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%, 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 penicilinase behaviour of the enzyme. The blaRUB-1 gene was chromosomally-located as revealed by I-CeuI-experiments and no gene homologous to ampR regulatory genes was found upstream of blaRUB-1 gene, which fits the non-inducibility of β-lactamase expression in *S. rubidaea*. 5′RACE analysis revealed promoter sequences that diverge from *E. coli* consensus sequence, thus explaining the low-level of expression in *S. rubidaea*.
Conclusions: This work further illustrates the heterogeneity of β-lactamases in *Serratia* spp. that may reflect a greater than expected variability of *Serratia* species.

P1214 Biochemical study of a new β-lactamase inhibitor enzyme (SHV-84) produced by a clinical *Escherichia coli* strain

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Objectives: Inhibitor resistant (IR) β-lactamases are mostly derived from parental TEM by point mutations in the corresponding coding gene. IR-SHV β-lactamases have been identified, mainly in *Klebsiella pneumoniae* strains. The aim of this study was the phenotypic, molecular and biochemical characterisation of a new IR-SHV enzyme – SHV-84 – produced by a clinical *Escherichia coli* strain.
Methods: *E. coli* INSRA4590 was isolated from a patient hospitalised in 1999 at a Portuguese hospital. Antimicrobial susceptibility was determined by an agar dilution method. β-lactamase encoding genes were screened by PCR and identified by nucleotide sequencing, using specific primers. Transformant *E. coli* DH5a harbouring the recombinant SHV-encoding plasmid was obtained by electroporation. SHV-84 was extracted from an overnight culture and purified by ion exchange and gel filtration. The kinetic constants were obtained by a computerised microacidimetric method. Isoelectric point of the β-lactamase was estimated by isoelectrofocusing.
Results: *E. coli* INSRA4590 was highly resistant to the penicillins tested (MICs of 512 to >4096 mg/L) and susceptible to cephalosporins and monobactam. This strain was resistant to the amoxicillin plus clavulanic acid combination (MIC of 64 mg/L), but remained susceptible to the piperaciclin plus tazobactam (MIC of 4 mg/L). The SHV-84-producing clone exhibited a β-lactam resistance phenotype similar to the clinical strain. Both produced an enzyme of pI of 7.4. The blaSHV-84 gene differed from blaSHV-1 by two point mutations that lead to the amino acid substitution Lys234Arg. SHV-84 showed a lower affinity (Km, 64 to 101 uM) for penicillins and cephalotin (Km 169 uM) than that of SHV-1 (Km, 11 to 31 uM and 40 uM, respectively). No hydrolysis was detected against extended-spectrum cephalosporins. The IC50 for SHV-84 was 2.21 uM for clavulanic acid, 13-fold higher than for SHV-1 (0.17 uM).
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 Lys234Arg amino acid substitution, 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.

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?

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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 oxyimino-cephalosporins without clavulanic acid synergy (n = 402) 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 blaampC sequence typing, antimicrobial susceptibility testing for β-lactam and non-β-lactam antibiotics by Etest and Vitek2, respectively. Moreover, characterisation of the genetic environment of blaampC, 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 blaCMY-2 (n = 35), blaCMY-7 (n = 1) and blaDHA-1 (n = 2), from out- (n = 23) and in-patients (n = 15) expressing moderate to high MICs for all β-lactam substrates, except cefepime and carbapenems. All isolates were co-resistant to trimethoprim-sulphamethoxazole and 58% expressed multidrug-resistance. Thirty-two (91%) blaCMY-2 and one blaCMY-7 were linked to ISEcp1 upstream and one blaDHA-1 (50%) was linked to qacEΔ1sul1 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), CC448 (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. BlaCMY-2 was seen carried on transferable multiple-replicon plasmids dominated by I1 (n = 12), FII (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.

Conclusion: CMY-2 is the dominant PABL among Norwegian *E. coli* isolates. It is strongly associated with ISEcp1 upstream and identified on multiple-replicon IncI1, IncA/C or IncFII transferable plasmids. The occurrence of PABLs in Norway is sporadic and is probably linked to globally dispersed uropathogenic 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

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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 b-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 b-lactamase production. Detection of resistance genes and integrases 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 b-lactamase was performed by hybridisation of I-CeuI/S1-PFGE.
Results: The isolates were MDR (predominant phenotype: amoxicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline) and harboured tetB, catA1, sul1, sul2 and blaOXA-30. The integron-borne OXA-30 β-lactamase was located on a conjugative plasmid classified as IncFIIA (140Kb), a common replicon among *Salmonella* virulence plasmids. Recently, in 2008, we identified from a hospitalised patient with gastrointestinal infection a clinical isolate also carrying the blaCMY-2 gene that was responsible for resistance to most of the large spectrum β-lactams, with exception for cefepime and carbapenems. The blaCMY-2 gene was located on a conjugative IncI1 plasmid of 75 kb and analysis of its genetic environment revealed blaCMY-2-blc-sugE genes downstream of a post-segregational killing system (pnd genes).
Conclusion: We described for the first time in Portugal the acquisition of a resistance plasmid carrying blaCMY-2 gene by a established MDR blaOXA-30-producing *Salmonella Typhimurium* clone, conferring resistance to therapeutically important broad-spectrum b-lactams. Moreover, the finding of blaCMY-2 gene in a conjugative plasmid IncI1 with a maintenance system is worrisome as persistence of this β-lactamase and its emergence in other *Salmonella* strains could be anticipated.

P1217 Prevalence of ampC β-lactamase genes in Enterobacteriaceae clinical isolates from Aveiro, Portugal

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Objectives: AmpC β-lactamases are enzymes that confer resistance to most β-lactams except carbapenems, thus are clinically relevant. The general mechanism for overexpression of chromosomally encoded AmpC β-lactamases is well known, however little is known about the occurrence of plasmid-borne ampC genes. In the present study, we investigated the prevalence of these plasmid encoded genes in clinical isolates collected in Aveiro, Portugal.
Methods: Clinical isolates of *Escherichia coli* (n = 30), *Citrobacter freundii* (n = 6) and *Klebsiella* peneumoniae (n = 50) were collected from different inpatients at the Hospital Infante D. Pedro EPE, Aveiro. Strains were selected based on a cefoxitin MIC >16 ug/ml and were tested with Etest (CN/CNI) (AB Biodisk) AmpC strips, according to manufacturer's instructions. ampC gene families and respective genetic environment were detected by PCR and amplified products were sequenced and compared with others deposited in the GenBank, by standard methods.
Results: Two different types of ampC genes were found, blaDHA-1-like in 5 *K. pneumoniae* and blaCMY-2-like in 6 *C. freundii*. As expected, all the blaCMY-2-like genes were linked to ISEcp1. blaDHA-1 genes have been found between ISCR1 and 3-CS2 of class 1 integrons in regions of variable length that may include qnrB.
Conclusions: Two different types of plasmid borne ampC genes were found among the population studied however, these results can only explain a small proportion of cefoxitin resistant phenotypes. blaCMY-2-like and blaDHA-1-like genes appeared in genetic contexts similar to those described by other authors. AmpC producers are a major concern in nosocomial infections and should be monitored by surveillance studies. These data highlights the importance of the identification of microorganisms producing plasmid encoded AmpC β-lactamases.

P1218 First detection of plasmid-mediated AmpC-type ACC-4 β-lactamase from *Proteus mirabilis* in Hungary

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Objectives: In August 2007 two clinical isolates of *Proteus mirabilis* showed 3rd generation cephalosprins resistance were isolated at an university teaching hospital and submitted to the National Center for Epidemiology to determine the mechanism of their resistance. Characterisation of these strains was performed.
Methods: The two clinical isolates were identified using the Micronaut E system (Genzyme Virotech GmbH). The MICs of ceftazidime, cefotaxime, cefepime, cefoxitin, imipenem, gentamicin, amikacin and ciprofloxacin 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, blaBIL, blaDHA, blaACC, blaMIR, blaACT, blaFOX 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 ceftazidime (>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 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 host. The plasmid cured strains became susceptible to ceftazidime (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.
Conclusion: 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*.

P1219 A complex outbreak of multiresistant Enterobacteriaceae producing the acquired AmpC-type β-lactamase FOX-7 in a neonatal intensive care unit setting

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Background: Enterobacteriaceae are a leading cause of infections in neonatal intensive care units (NICUs). Plasmid-encoded AmpC-type β-lactamases (pACBLs) are resistance determinants of increasing clinical relevance in Enterobacteriaceae. Production of these enzymes is tipically

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 pACBL 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*, 2 of *K. oxytoca* and 2 of *P. agglomerans*. Molecular analysis showed carriage of the FOX-7 pACBL gene in all the 27 isolates. PFGE genotyping showed a multiclonal nature of the *K. pneumoniae* isolates (three different clones). A similar outbreak had been reported in the same NICU during 2003–2004. Analysis of representative isolates (three *K. pneumoniae* and one *K. oxytoca*) from that episode confirmed the presence of the FOX-7 pACBL but revealed clonal diversity compared with the 2008 isolates.
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.

P1220 Phenotypic and genotypic analysis of a novel extended-spectrum β-lactamase phenotype (cefepimease)

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Objectives: To characterise an unusual ESBL phenotype ("cefepimease") observed in 18 isolates of *S. Typhimurium*. The phenotype is characterised by high cefepime MIC (relative to other cephalosporins) that is markedly reduced by clavulanic acid.
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 for 31 β-lactamase encoding genes by PCR using specific primers. Sequencing of amplicons was performed in both directions using primers homologous to those used for PCR. Isoelectric focusing (IEF) was performed using previously reported protocols. An association between blaOXA-1 and Insertion Sequence Common Region I (ISCR1), IS1, IS26, ISEcp1 was investigated by PCR. Cloning of OXA into the pBK-CMV vector and subsequent transformation into XL1 cells was performed. Plasmid extraction was performed by an alkaline lysis method. Transfer of cefepime resistance was attempted using solid, liquid and filter mating methods with *E. coli* J53 AzR as recipient. Typing was carried out by PFGE (using XbaI and Bln1) and VNTR.
Results: All isolates were multidrug resistant with 4 antibiograms observed. PFGE using Xba1 and Bln1 and VNTR analysis identified 14, 8 and 13 profiles respectively. Each isolate harboured 2 to 5 plasmids ranging 8.6 kb to 135 kb. blaTEM-1 and blaOXA-1 were the only β-lactamase encoding genes identified and β-lactamase enzymes of corresponding isoelectric point's (pI's) of 5.2 and 7.2 were observed in all 18 isolates. Class 1 integrons of 800 bp or 2000 bp were observed in all isolates. Class II integrons were not detected. A link between OXA-1 and ISCR1 was observed in all isolates. Transfer of cefepime resistance by conjugation was not achieved. Cloning of blaOXA-1 into pBK-CMV and transformation into XL1 cells did not result in expression of the 'cefepimase' phenotype.
Conclusions: This unusual phenotype of resistance is present in a genetically diverse group of *S. Typhimurium*. The identified bla genes do not account for the observed phenotype. OXA-1 effectively hydrolyses cefepime but is not markedly inhibited by clavulanic acid and transfer of the blaOXA-1 gene into *E. coli* did result in the "cefepimase" phenotype. The genetic basis for the resistance is not yet fully characterised but may be related to expression of OXA-1 in the specific genetic context of these strains.

Central nervous system infections

P1221 Implementation of adjunctive dexamethasone improves outcome in pneumococcal meningitis

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Objective: 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 thrombocytes 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.

P1222 Central nervous system involvement is common in acute Puumala virus infection

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Objectives: Puumala virus (PUUV) causes a haemorrhagic fever with renal syndrome called nephropathia epidemica (NE). Recent case reports and retrospective studies have revealed a potential connection between acute NE and central nervous system (CNS) complications. Viral meningitis, encephalitis, cerebral haemorrhage and hypophyseal injury, for example, have been reported. Based on these recent observations, our goal was to explore the frequency and nature of such complications prospectively in patients with acute NE.
Methods: 58 patients with serologically proven acute NE volunteered for the study at Oulu University Hospital during 2005–2007 (approval by ethical committee of the Oulu University Hospital). CNS symptoms were recorded and urine, blood, and cerebrospinal fluid (CSF, 42 patients) samples were collected. The patients underwent magnetic resonance

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.

P1223 In vitro comparison of bacterial permeability of different epidural catheter filters

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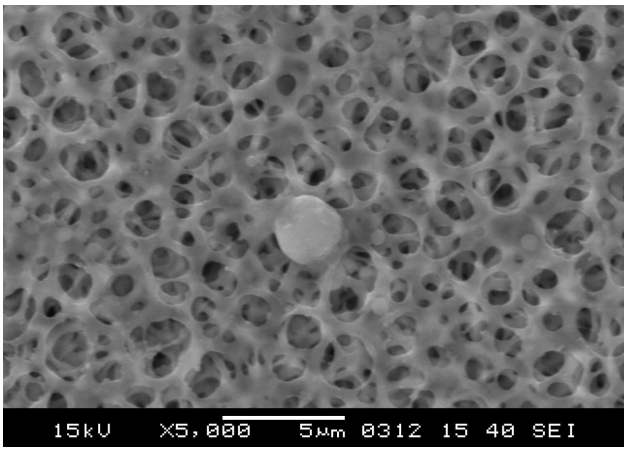
Objectives: The rate of infection occurrence is approximately 5.4% during epidural analgesia/anaesthesia administration. Requirement for bacteria filters to reduce the infection risk is one of the most debated topics. Conflicting results have been reported for the effectiveness of different filters for 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

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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 $\geqslant$ 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

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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 (Tunkel-AR 2004, BIS algoritme 2004 (similar to Fitch-MT 2007), van Crevel-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

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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

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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 $\pm$0.45) than in the non-meningitis group (3.6 g/dL $\pm$0.6) ($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

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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.1.14 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

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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

Infectious Diseases Department of the University Hospital Bulovka in Prague, Czech Republic, in years 1997–2008.

Results: Twenty-six patients with *L. monocytogenes* meningitis were hospitalised, what is 7.5% of the total of 348 adult patients with community-acquired bacterial meningitis hospitalised within 12 years. They were 12 women and 14 men with the median age of 63 years, range 26–80 years. In all patients *L. monocytogenes* was identified by culture: CSF was positive in 21 pts, blood in 11 pts and autopsy meningeal tissue sample in 1 patient. Predisposing factors were major immunocompromise (HIV, leukaemia, lymphoma, long-term corticosteroids) in 9 pts (35%), age higher than 60 years in 17 pts (65%), minor immunocompromise (diabetes, alcoholic liver disease, autoimmunity) in 14 pts (54%) and arteriosclerosis in 16 patients (62%). All patients presented with fever, 88% with disturbed consciousness, 79% with headache, 69% with positive meningeal signs, but only 25% with vomiting and 16% with seizures. The median count of CSF polymorphonuclears was 790/cmm and lymphocytes 182/cmm, protein level 2.7 g/L and glucose ratio 0.25. Mononuclear cell predominance was present in 19% of patients. Fifteen patients (58%) recovered with no or mild sequelae, 6 pts (23%) with moderate sequelae, 1 patient (4%) with severe sequelae and 4 pts (15%) died.

Conclusion: Besides major immunocompromise and elderly also minor immunocompromise and arteriosclerosis should be considered as predisposing factors for *L. monocytogenes* meningitis in adults. While there are no prominent differences in clinical manifestation and outcome between Listeria meningitis and other bacterial meningitis in adults, in laboratory parameters CSF leukocytes tend to be lower in Listeria meningitis.

Pathogenesis of infections caused by Gram-negative bacteria and mycobacteria

P1230 Fibronectin stimulates *Escherichia coli* phagocytosis by microglial cells

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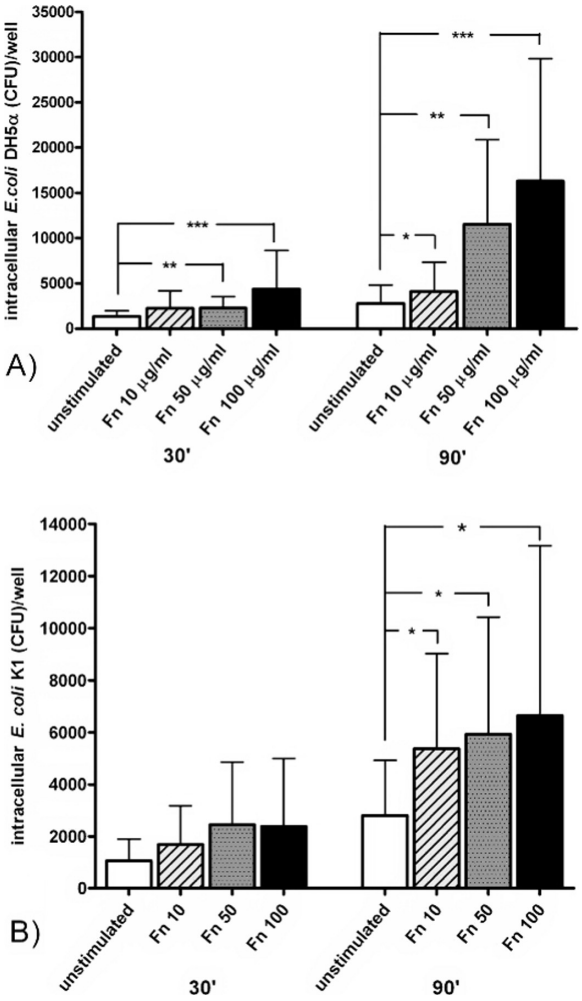
Objectives: Infections caused by *Escherichia coli* are common in the clinical setting and are still associated with high rates of mortality and long term sequelae despite antimicrobial therapy. Parenchymal microglia are one of the effective defence systems in the brain to remove invading bacteria contributing 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*.

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 −80ºC 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 at 37ºC. For phagocytosis inhibition studies, cytochalasin D (CD) was used at 10 microM. After bacterial exposure, microglial cultures were washed and incubated in medium containing gentamicin (200 mg/l) for 1 h to kill extracellular bacteria. Thereafter, cells were washed and lysed 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 ($n \geqslant 12$); $p < 0.05$ 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 $\geqslant$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*.



P1231 A recombination hotspot with several genomic islands and a high number of putative pili operons in the chromosome of an epidemic *Enterobacter hormaechei*

A. Paauw, M.A. Leverstein-van Hall, J. Verhoef, A.C. Fluit (Utrecht, NL)*

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 contigs was reduced. The remaining contigs were concatenated with a recognition sequence between the contigs. The concatenated genome sequence was annoted by the JCVI Annotation Service. Overrepresentation of each cluster of orthologous groups (COG) in the EHOS was determined by comparing the COG distribution of the EHOS with the COG distribution of 10 other Enterobacteriaceae.
The possibility of excision and circularisation of the HPI was investigated by PCR using primers at sites flanking all attO repeats. Obtained products were sequenced.
Results: The chromosome consisted of approx. 4,857 kb with a GC content of 55.4% and 4,645 open reading frames (orfs) were defined. A relatively high number of these orfs encoded proteins supposedly involved in cell wall/membrane biogenesis (5.1%) and cell motility (2.9%). The EHOS contained 18 operons putatively encoding 15 different pili and one type of flagella. Based on the presence of attO repeats, 5 genomic islands located next to each other were identified of which one contained the HPI. The function of the other genomic islands is unknown or speculative. The genomic islands could be excised from the chromosome in different combinations demonstrating the plasticity of this region and therefore designated a recombination hotspot (RHS).
Conclusions: The genome of the EHOS contained 1) operons that putatively encoded 15 different pili and 2) a RHS that contained 5 genomic islands, one containing the HPI. The pili, the RHS, and the HPI are likely important factors in niche adaptation which may have contributed to the epidemic behaviour of the EHOS.

P1232 The siderophore yersiniabactin produced by the high-pathogenicity island of Enterobacteriaceae reduces the oxidative stress response of polymorphonuclear leukocytes

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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: Growth experiments with the EHOS and the HPI-knockout showed that 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 tnpA gene. This new variant was designated HPI-ICEEh1.
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-ICEEh1, which showed a high similarity to HPI-ICEEc1.

P1233 The genotoxin producing *E. coli* in the intestinal microbiota; a new view on commensalism

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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 genes may be located in particular regions on the bacterial chromosome termed pathogenicity islands such as the pks island carried by certain phylogenetic B2 *E. coli* strains. Contact with *E. coli* strains expressing the pks island causes 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 sampled from colonic 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 phlygenetic 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

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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 diarrhoeal 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 Cravioto 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 dit 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 24 hrs 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 acumulated 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 prophylaxis, but even in the treatment of gastro-intestinal chronic disorders, as an alternative or in association with antibiotics.

P1235 Low virulence associated with fluoroquinolone resistance of uropathogenic *Escherichia coli* strains

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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 flouroquinolone
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 bacteriuria. 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 adhesins 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 trimethoprim/sulfametoxazole, 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 adhesins expression 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*.

P1236 Endemic clone *Escherichia coli* harbouring ECP common pillus versus an outbreak clone in a patient from a hospital, Lisbon

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Objectives: The aim of this study was to compare an endemic *Escherichia coli* producing CTX-M-15 extended-spectrum b-lactamases (ESBL) with an outbreak clone *E. coli* producing AmpC β-lactamase, which is maintained during only one month.

Methods: Four *Escherichia coli* isolates were identified in a period of three years, from an old patient hospitalised twice times at Hospital Santa Maria in Lisboa. The first one collected in August 2001 from urine, two isolates from urine and blood in April 2004 and the fourth isolate was identified from urine one week later. Antibiotic resistance profiles were determined by disc diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) and the β-lactamase genes were identified with specific primers and sequencing. RAPD M13 DNA fingerprint and phylogenetic group was determined in order to obtain genetic profiles. PCR reaction was also performed to detected genes encoding virulence factors, including adhesins of type P (papC), 1 (fimH) and *S. fimbriae* (sfaS), cytotoxic necrotising factor-1 (cnf-1), siderophore biosynthesis protein (iucC), α-haemolysin (hlyA), uropathogenic specific protein (usp) and also a subunit of ECP common pillus (ecpA).

Results: All three isolates identified from urine in August 2001 and three years later from urine and blood in 12 April 2004 were ESBL producing strains (CTX-M-15), exhibit the M13 fingerprinting profile 1, the most prevalent in this hospital, belonged to the phylogenetic group B2, typically associated with virulence strains, and harbour fimH, iucC and ecpA genes. The fourth isolate, an outbreak strain identified from urine eight days after (20 April 2004), exhibit a distinct genetic profile. This strain harboured a different β-lactamase, the cephalosporinase AmpC, exhibited the M13 fingerprinting profile XY, included in phylogenetic group B1, usually associated with commensal strains and the virulence genes were not detected including the subunit of ECP common pillus (ecpA).

Conclusions: The endemic clone *E. coli* producing CTX-M-15 was prevalent in Hospital since 2001 until present. ECP common pillus allows the human intestine colonisation and it might confer an evolutionary advantage, contributing to maintenance and higher prevalence of this clone during three years in this patient compared with outbreak clone *E. coli* producing AmpC β-lactamase, which was detected in four wards, only during one month, in this hospital.

P1237 Potential role of *Escherichia coli* common pilus in persistence of clinical isolates producing extended-spectrum β-lactamase from bacteraemia and urinary tract infection

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Objectives: The aim of this study was to investigate the role of *E. coli* common pilus (EcpA) in extraintestinal *E. coli* producing extended-spectrum β-lactamases that carrying virulence factors and are responsible by bacteraemia and urinary tract infection (UTI).

Methods: *E. coli* clinical isolates identified from urine ($n = 103$) and blood ($n = 70$) were collected between 2001 and 2007 at Hospital Santa Maria, in Lisboa. In this study were included 26 simultaneous clinical isolates recovered from 12 patients with both urine and blood sample RAPD M13 DNA fingerprint and phylogenetic group was determined in order to obtain genetic profiles. PCR reaction was also performed to detect a subunit of ECP common pillus (ecpA). The Fisher's exact test was used for categorical variables, $P < 0.05$ statistically significant.

Results: The majority *E. coli* strains belonged to the phylogenetic group B2 (62.5% urine Vs 65.7% blood) and group D (17.5% urine Vs 18.5% blood). Surprisingly was the fact that equal frequency ($p < 0.8036$, 95IC0, 6663–1.836) was detected to commensal phylogenetic group A (15.0% urine Vs 12.8% blood), usually associated with low invasive

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 bacteriaemia showed the ecpA gene.

Conclusions: Bacteraemia 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 bacteraemia. 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

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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 urease-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 provided 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 – chondroitin sulfates, 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 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 adherence. Chondroitin sulfate C (ChSC) and A (ChSA) significantly increased crystals binding to epithelium.

Conclusion: Urinary glycosaminoglycans had no inhibitory effect on infection-induced crystallisation. Our results showed that ChSC and CHSA promoted crystal growth and the retention of crystals in the urinary tract which increased the formation of urinary stones.

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P1239 Heat shock-induced phage shock protein A increases *Salmonella typhimurium* virulence in BALB/c mice

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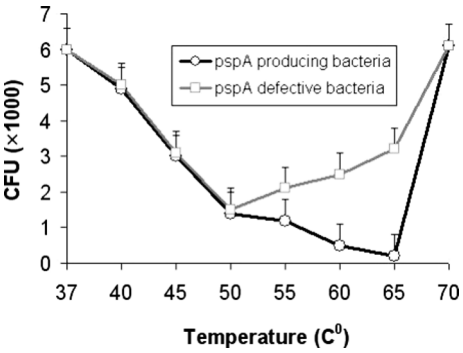
Objectives: *Salmonella typhimurium* is an intracellular pathogen that bring about thousands reported cases of acute gastroenteritis and diarrhoea each year. Although many successful physiological and genetic approaches have been taken to conclude the key virulence determinants encoded by this organism, 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 (10 ml) of working cultures (10^8 CFU/ml) were heat stressed by immersion (3 cm above medium level in bottle) at 40ºC, 45ºC, 50ºC, 55ºC, 60ºC, 65ºC and 70ºC 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 cells.

Results: Lethal dose of untreated *Salmonella typhimurium* for 50% of female 6–8 weeks old Balb/c mice inoculated orally was 6×10^3 CFU. At the point of 65ºC; 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*.



A sixteen-fold difference between LD50 s of PspA producing and non-producing *Salmonella typhimurium* cells to Balb/c mice.

P1240 *Vibrio* species utilise *Acanthamoeba castellanii* as an environmental host

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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 septicaemia. 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. Gentamicin assay was used to kill extracellular vibrios as well as to examine ability of amoeba to protect intracellular vibrios from antibiotic killing. Interaction between

microorganisms was studied by viable count, necrosis assay, fluorescence microscopy, electron microscopy and statistical analysis.
Results: The results showed that *Acanthamoeba castellanii* grew in the presence of *Vibrio* species and the amoeba numbers enhanced during 2 weeks.
Growth of the bacterial strains was enhanced significantly in the presence of *A. castellanii* compared to alone cultivated bacteria, which died within few days.
The examined vibrio species grew intracellularly to 10^4–10^6 CFU/ml in *A. castellanii* and the intracellular bacteria survived for >2 weeks.
Electron microscopy showed that the intracellular localisation of the bacteria was in vacuoles of the trophozoites a few hours after co-cultivation. Multiplication of bacterial cells occurred in the cytoplasm of trophozoites one day after co-cultivation and the bacteria were found in the cysts of *A. castellanii* 6 and 7 days after co-cultivation.
Conclusions: *Vibrio cholerae*, *V. mimicus* and *V. vulnificus* grew and survived in *A. castellanii* disclosing the role of acanthamoebae as environmental hosts for *Vibrio* species.

P1242 In vitro study of dendritic cells maturation induced by *Helicobacter pylori* strains: evaluation of the inflammatory response and immunological consequences

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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 antibody array and ELISA. The ability of *H. pylori*-activated DCs to induce allogenic 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, MIP1-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 and IL-23 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.

P1243 Relationship between prevalence of peptic ulcer and cagA/iceA genotypes of *Helicobacter pylori*

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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 Iranien 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.

P1244 *Helicobacter pylori* genetic variability and multi-drug resistance in a case of gastric cancer

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Objective: *Helicobacter pylori* is involved in chronic gastritis, gastro-duodenal ulcers, gastric cancer and MALT lymphoma development. The virulence genes vacA, iceA and cagA play a key role in the onset of the infection. In particular, *H. pylori* strains possessing the cagA gene with repeated sequences containing EPIYA motifs are associated to severe gastroduodenal diseases. We report a case of a patient colonised by *H. pylori* and affected by gastric cancer. Multiple *H. pylori* strains, possessing the main virulence factors and displaying a different antimicrobial susceptibility pattern were harboured in the same host.
Methods: A 68 years old female patient, identified among a group of individuals subjected to upper gastrointestinal (GI) endoscopy for gastrointestinal compliances and previously subjected to an anti-*H. pylori* therapy, was examined in this study. Two biopsies, collected from the antrum, were analyzed for: *H. pylori* culture, genetic variability through Amplified Fragment Polymorphism (AFLP); vacA, iceA, cagA virulence status by PCR; antimicrobial susceptibility (CMI) towards the antibiotics commonly used in anti-*H. pylori* therapy and for hystopathological examination. A total of 20 clones were analyzed.
Results: The patient with gastric cancer showed a mixed infection with the presence of at least 3 different strains. The clones isolated possessed the main virulence factors (vacA s1 m1, iceA1, cagA EPIYA P1P2P3) and a pattern of multi-drug resistances were evidenced in the different clones.
Conclusion: The data obtained in this study confirm the correlation between the severity of the disease and the presence of a greater number of cagA EPIYA motifs. In particular, the presence of multiple *H. pylori* strains colonising the same patient, with the main virulence factors and different multi-drug resistance among isolates, point out the role of genetic variability generating, in time, more virulent and adapted strains. All these data emphasize the need for a careful *H. pylori* antimicrobial surveillance to improve management of *H. pylori* infection.

P1245 **Circulating plasmablasts with reactivity against individuals' own intestinal microbiota in patients with acute appendicitis**

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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 polymicrobial 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.

P1246 **Cloning and characterisation of EngA, a GTP-binding protein from *Mycobacterium tuberculosis* H37Rv**

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Guanine nucleotides are critical elements and key signaling molecules. Many members of 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. These proteins act as molecular switches and modulate between GTP bind (active) and GDP bind (inactive) conformations. These proteins are known to play diverse roles in the life cycle of both prokaryotes and eukaryotes. Comparative genomic approach was used to predict homologs of GTPase in *Mycobacterium* genome. Amino 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 DXXG motif, which is a characteristic feature of all known GTPases is also conserved in the EngA of *M. tuberculosis* H37Rv. In the present study, 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

P1247 **One-year perspective study on prevalence and characterisation of diarrhoeagenic *Escherichia coli* isolated from children, beef and cattle in Tehran, Iran**

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Objective: Several routes exist for human infection with Diarrhoeagenic *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.
Method: From November 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 reffered to Tehran children's hospital were tested. All samples were investigated and identified for DEC and their virulence genes (stx1, stx2, eae, Lt, St, ial, ipaH, bfp, ttc, 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 (STEC). 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 gen (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, lt/st, ipaH, ial and Pcvd432 genes in stool specimens of children respectively. SOD gene was positive in 18.6%, 17.3% and 37.43% of STEC strains, wich are detected from Children, Meat and Cattle faeces respectively. Bloody diarrhoea 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.

P1248 **Bacterial causes of gastroenteritis in the Netherlands: ten-year trends**

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Objectives: To study the epidemiology and time-trends of bacterial gastroenteritis.
Methods: Analyses of all routine stool cultures performed from 1997 up to 2006 in a laboratory covering a catchment population of almost one million persons. Antibiotic sensitivity of detected pathogens was tested according to NCCLS criteria.
Results: A total of 105,179 stool samples were cultured. Of these 9.7% was positive with *Campylobacter*, *Salmonella*, Shighella or *Yersinia* species, or *Escherichia coli* O157. Of all positives 12.6% appeared to be (a) repeat culture(s). *Campylobacter* and *Salmonella* species were the most prevalent pathogens, found in 5.7% and 3.8%, respectively. In 0.9% of the positive specimens more than one pathogen was detected. Women sent in more samples than men (56% versus 44% of total), but men were more often positive than women (11.2% versus 8.6%). The highest proportion of positive cultures was found in age-category 10 to 20 years (14.7%), the lowest in under 1 and over 80 years (5.6% and 3.8%, respectively). February was the month with lowest and August with highest number of positive cultures (465 and 1402, respectively).
Over time, the number of samples for culture remained constant. There was a clear trend of decreasing incidence of *Salmonella*, from 457 newly positive persons in 1997 to 221 in 2006. The decrease was most pronounced in age group zero to five: 154 new cases in 1997 to 51 in 2006. In *Campylobacter* species a trend was observed of increasing fluoroquinolone resistance: from 25% in 1997 to 47% in 2006.
Conclusion: *Salmonella* infections are gradually decreasing, particularly in the under-five population. In 10 years time *Campylobacter* fluoroquinolone resistance doubled to almost half of all isolates.

P1249 Analysis of the gut microbiota of irritable bowel syndrome patients and healthy volunteers using selective media and Real-Time PCR

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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

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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 subtropics 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

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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 co-infection 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

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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 a 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.

P1253 Real-time comparison of *Listeria monocytogenes* PFGE profiles of human and food isolates for enhanced epidemiological investigation of listeriosis

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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 AscI-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.

P1254 The prevalence of diseases associated with *Helicobacter pylori* in St. Petersburg, Russia at present

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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 gastoduodenitis, 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 maximum in the 20–29 y.o. group and in 30–39 y.o. group which is correspondingly equal to 52.27% 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.

P1255 Gene mutations of 23S rRNA and rdxA deletion associated with clarithromycin & metronidazole resistance in *Helicobacter pylori* strains isolated from UAE patients

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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 extraxted 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 were as 22 by clotest. Resistance to clarithromycin and metronidazole was detected in 9 (34.6%) and 3 (11.5%) of 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.

P1256 Resistance to antibiotics of *Helicobacter pylori* strains from patients after treatment failure

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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 microrganism 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 oxydase, urease and catalase tests. Antibiotic susceptibility was tested by Kirby-Bauer and E-test for metronidazole (MZ), levofloxacin (LEV), tetracycline (TE), claritromycin (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 pangastritis). CLA and MZ showed the highest resistance: 56.26% (18 strains) and 71.87% (23 strains) respectively. Resistance to both AMX 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 used were observed except for MZ concerning 2 strains that showed resistance only by E-test. Out of the 11 patients with pangastritis, 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 CLA was found in 16 patients (64%). LEV appears a promising alternative for Hp refractory infections. A distinct pattern of antibiotic sensitivity of isolates belonging to different districts of the stomach (heteroresistance) was noticed in 22.2% of cases. The presence in the same patient of Hp strains either S or R to various antimicrobial agents, may interfere with the outcome of the therapy.

Table 1. Distribution of MICs (mcg/ml) and sensitivity, intermediate and resistance values for 32 *Helicobacter pylori* isolates from 25 patients under consideration.

Antibiotic	⩽0.5	0.5–15	2–3.5	4–7.5	8–32	48–128	⩾256	MIC cut-off	S, % (N)	I, % (N)	R, % (N)
MZ	0	2	4	3	16	4	3	⩾8	21.88 (7)	6.25 (2)	71.87 (23)
CLA	6	8	6	4	4	3	1	⩾2	40.62 (13)	3.12 (1)	56.26 (18)
TE	10	9	8	3	2	0	0	⩾4	75.00 (24)	9.38 (3)	15.62 (5)
AMX	16	10	3	3	0	0	0	⩾2	78.12 (25)	3.12 (1)	18.76 (6)
LEV	8	10	4	4	3	3	0	⩾8	81.24 (26)	0 (0)	18.76 (6)

Legenda: MZ = Metronidazole; TE = Tetracycline; LEV = Levofloxacin; CLA = Clarithromycin; AMX = Amoxicillin.

P1257 Different strains of pathogenic *Yersinia enterocolitica* distributed among wild boars and domestic pigs in Switzerland

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Objectives: Domestic pigs are an important reservoir of human pathogenic *Yersinia enterocolitica* in many countries including Switzerland. In the past years, wild boar population has increased considerable 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 (ail, yst, hreP, virF and yadA) were studied by PCR and the genotype was studied by PFGE using NotI, ApaI and XhoI 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 bioserotyep 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/sulfamethoxazole 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

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Objective: 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 221patients. 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 clindamycin)
- Decontamination of hands with soap & water

Results: Pre-outbreak typing (Jan and Feb 2006) showed 80% ribotype 001, 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 in to 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.

Conclusion: An outbreak of *C. 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.

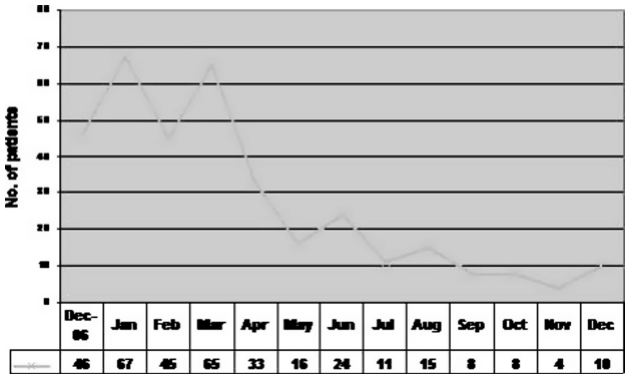

Figure: *Clostridium difficile* episodes – December 2006 to December 2007.

P1259 In vitro and in vivo comparison of *Clostridium difficile* PCR ribotype 027 and non 027 strains

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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

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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 cm^2) 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

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Objectives: To investigate the toxigenicity and the resistance to antibiotics of Cd strains isolated from hospitalised patients suffering from Cd 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 Cd 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 EIA (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: Cd 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 Cd 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

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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

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Objectives: The use of antimicrobials in production animals is a major cause of antimicrobial resistance in *Campylobacter* strains. Erythromycin, a macrolide group antimicrobial agent, is one of the drugs of choice for the treatment of human campylobacteriosis. Tylosin is a macrolide 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, nalidixid 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 SmaI).

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 $\geqslant$ 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

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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 infuencing 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

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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 AC11/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/mm^3. 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.

P1266 Performance of the BED-CEIA in population infected with HIV-1 recombinant viruses CRF06$_{cpx}$ and CRF06A

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Objectives: The Calypte HIV-1 BED Incidence EIA (BED-CEIA) was developed to overcome the HIV subtype dependence associated with many of the assays used in the identification of recent HIV infection. Indirectly, the assay determines the proportion of HIV-1 specific IgG to the total human IgG and has been used in populations containing subtypes B, D, E and C. However, the use of this assay in populations infected with recombinant viruses has not been described. To evaluate the assay performance in a population infected with HIV-1 recombinant viruses CRF06$_{cpx}$ and CRF06A and to determine the prevalence of recent infections among newly diagnosed subjects.
Methods: The blood sera were collected in subjects with the first positive HIV test but unknown negative test in the past (group A; n = 122), in those with the history of positive HIV test of more than 6 months ago (group B; n = 65) and in subjects with first positive HIV test and known HIV negative test within last 3 months (group C; n = 6); all nave to antiretroviral agents. The BED-CEIA was performed as per manufacturers instructions with the optical density (OD) values of test specimens normalised by a ratio using a calibrator to minimise interrun variation. As per instructions samples were tested in singleton but those with a normalised OD (ODn) ⩽1.2 were re-tested in triplicate and the median values were used for evaluation. An ODn value <0.8 was considered to indicate recent infection. The receiver operating curve (ROC) was constructed using ODn data of group B and C. The performance of curve was considered fair if the AUCROC value was between 0.7 and 0.8.
Results: The mean SD ODn values in subgroup A, B and C were 0.70 0.69, 0.86 0.68, and 0.32 0.19, respectively. A total of 87/122 (71%) subjects in group A, 31/65 (48%) in group B and all 6 in group C were categorised into recent infections. The calculated sensitivity and specificity with the NPV and PPV were 100% and 52%, 100% and 16%, respectively. The ROC curve constructed using ODn values of subgroup B and C showed an AUCROC value of 0.77 SD 0.08. No correlation between viral load and ODn value was observed.
Conclusion: In subjects infected with CRF06$_{cpx}$ or CRF06A viruses the BED-CEIA has good sensitivity but lacks required specificity. Whether the poor specificity of the assay is due to the viral subtype or associated with the frequent reinfections commonly seen in IDUs, or another reason remains to be identified in future trials.

P1267 Study on the genotypic resistance of HIV-1 in blood monocytes, CD4 T cells and plasma of HIV-infected individuals

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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+ monocyte, CD4+ T cells and plasma.
Methods: Plasma, CD14+ monocyte and CD4+ T cells were collected from 8 treatment-naive individuals and 33 treated-patients; of the latter, 10 showed undectable levels of viraemia and 23 were on virological failure. CD14+ monocyte and CD4+ T cells were isolated using magnetic beads for positive selection (Miltenyi Biotec). Genotyping of the reverse transcriptase (RT) and protease gene (pro) of HIV-1 was performed using fluorescent dideoxy-terminator method (TRUGENE HIV-1-Siemens Heathcare Diagnostics). HIV drug resistance was defined according to the HIV-1 genotypic resistance interpretation algorithm of the GUIDE LINESTM 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 undectable level of HIV-RNA (<50 copies/ml). The results obtained revealed that, in 80% of samples, the HIV drug resistant variant harboured 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-naive 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

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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 peformed 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).

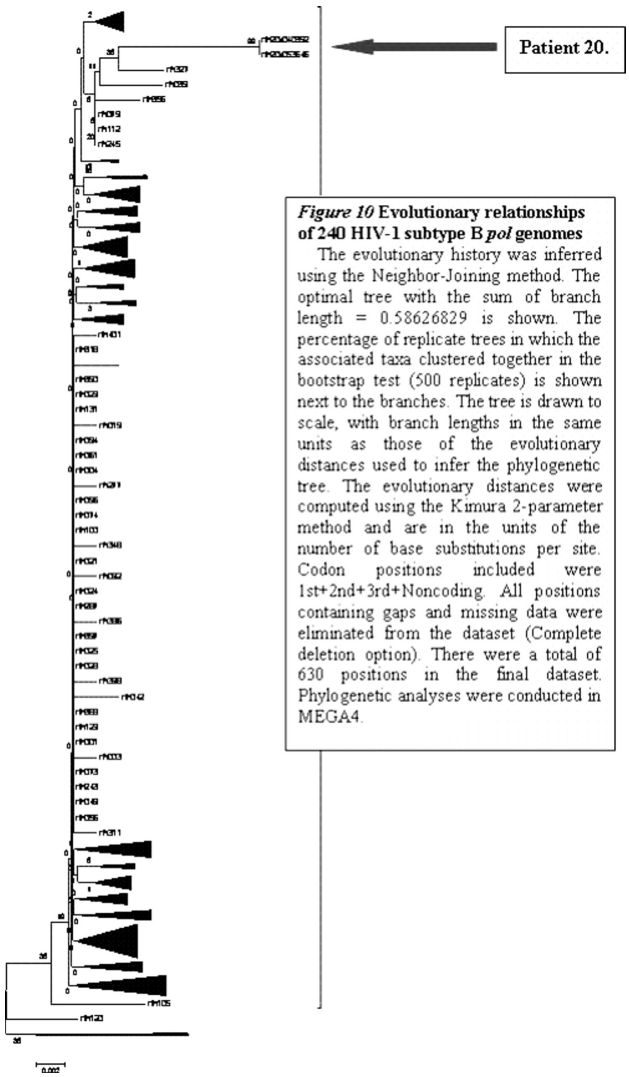

Figure 10 **Evolutionary relationships of 240 HIV-1 subtype B *pol* genomes**

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.58626829 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 630 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4.

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

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Background: The multifactorial mechanisms by which some HIV+ 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+ naive patients with different degrees of progression and viral load (VL) were analyzed, of them 34 were LTNP from the HCIII cohort (mean infection: 18.62 years; mean CD4: 657 cells/ul) and 18 were progressors. Several host genes were analyzed in DNA from PBMCs: CCR5 and HLA were typed by PCR. HLAC rs9264942 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, HLACw1203 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 HLACw04 and Cw07 ($p < 0.0005$).

Conclusion: HLA plays an important role in HIV infection control in our group of patients due to HLA-B5701, 2705 and HLACw1203 genotypes were associated with non-progression. The presence of HLA-C rs9264942 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.

HLA	Prog (%)	LTNP (%)	P value
HLA B*5701	0/16 (0)	8/29 (28)	0.04
HLA B*2705	0/16 (0)	7/29 (24)	0.04
HLA Cw0401	5/16 (31)	7/30 (23)	0.73
HLA Cw0701	5/16 (31)	6/30 (20)	0.48
HLA Cw1203	0/16 (0)	8/30 (27)	0.04

P1270 Knowledge on sexually transmitted infections of HIV-infected patients

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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 significative) 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±6.0 y, most of them actually on treatment (75%); mean self reported CD4 cells were 535.2/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 y), lower education level (primary vs secondary school or university), unemployment (vs employment) and suppressed viraemia (HIV RNA $\log_{10}$ <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.

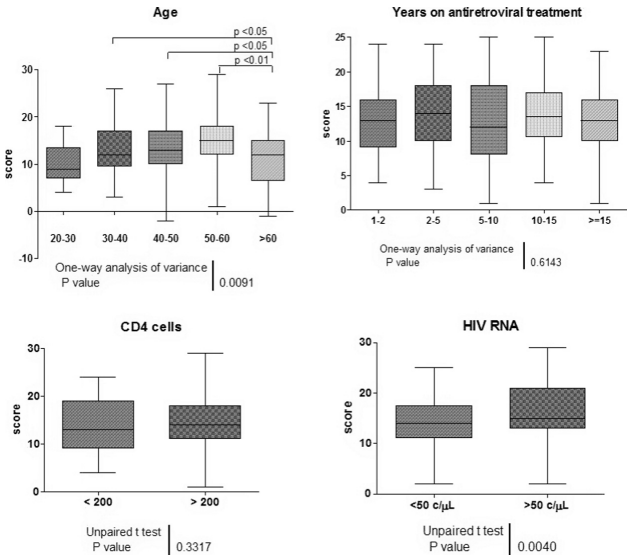

Figure: Scores of knowledge on HIV/STIs according to age, years on antiretroviral treatment, CD4+ cells and HIV viraemia.

P1271 Prevalence of HIV-infection in Saudi Arabia

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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 ranging 229–342 cases per year on 2001–2006. Between 2001 and 2006 the mean 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.

P1272 Mother-to-child transmission of HIV: a Portuguese retrospective study

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Objectives: Mother-to-child transmission of HIV is the most important type of HIV infection in childhood. We analysed if the use of antiretroviral (ARV) drugs reduced the mother-to-child transmission of HIV and compared the results with other studies done before.
Methods: HIV infected pregnant women and their newborns, born during from January 1992 through December 2005, followed at Hospital Joaquim Urbano were included. Demographic, maternal, obstetric, clinical, therapeutical data were collected retrospectively for the mother-child pairs.
Results: Of the 158 mother-child pairs, 150 were enrolled. HIV1 was present in 94.7% mothers, HIV1+2 in 2–0% and HIV2 in 3.3%. Heterosexual acquisition of infection was registered more often (56.7%) than injecting drug use (39.3%). Diagnosis of HIV infection was mainly during pregnancy (42.7%)and most mothers were asymptomatic. Transmitter mothers knew their HIV status mainly during delivery (57.1%) or after birth (28.6%) ($p < 0.001$) and arrived later at health care system ($p = 0.048$). Their average CD4 cell count was lower and their HIV level was higher. The use of ARV during pregnancy (OR:55.5) and in the three parts (OR:58.5) were associated with a lower transmission of HIV when compared to the absence of ARV ($p < 0.001$). Infected children weight less (73.8% were <2500 g) ($p = 0.045$) and most of them were born vaginally (N = 13/14). Vaginal delivery and breastfeed were associated to HIV transmission ($p < 0.001$ e $p < 0.05$, respectively). The rate of transmission was 9.3%. This rate has decreased through the study years [<1994: 25%, 1994–1998: 4%, >1998: 6.8%] and after 1998 only three mothers didn't use ARV – two infected newborns. After delivery, mothers stopped the use of ARV and their RNA-HIV levels had rised.
Conclusions: We found that an early diagnosis of HIV infection in young and pregnant women, an early follow-up by the health system, the use of antiretroviral drugs in pregnancy, intrapartum and in the newborn, caesarean section and exclusion of breastfeed of the child were associated with a reduction of mother-to-child HIV transmission. The low birth weight of the newborn was found to be a factor of bad prognosis for HIV infection. Our results suggest that the use of ARV drugs in pregnancy,

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

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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 lipoathrophy and mitochondrial toxicity among non-Caucasian patients treated at an AIDS centre, Jerusalem

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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 became a major parameter when antiretroviral regimen is prescribed.
Lipo-atrophy 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 Abacvir/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-Caucasion 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 log10mean 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

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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-B5715 and HLA-B5716. 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

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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

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 (85.7%) 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, and 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 Cockroft-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

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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 (89%) 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 md/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 md/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 ($\beta = 0.844$, $p < 0.001$). 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

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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 immunological responses.

P1279 Serological screening of Chagas' disease in HIV-positive immigrants proceeding from endemic areas in Spain

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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, oesophagogastroscopy, barium enema, and echocardiography was applied.
Results: We screened 19 HIV positive immigrants (mean age 36 years). The procedence 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.

P1280 Similar aortic stiffness but decreased wave reflections in human immunodeficiency virus-infected naive patients. The role of subclinical inflammation

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Background: The pathogenesis of increased cardiovascular risk in human immunodeficiency virus (HIV) infected patients and the role of HIV infection, per se, as a risk factor for accelerated arteriosclerosis remains questionable. Our aim was to investigate the impact of HIV infection on vascular properties along with markers of subclinical inflammation in HIV naive (untreated) patients.
Methods: We studied 55 HIV-infected naive patients but without AIDS (aged 33 years, 52 males, 30 smokers) and 31 healthy individuals matched for age, sex and smoking status. Aortic augmentation index (AIx) and augmented pressure (AP) were assessed using applanation tonometry of the radial artery. Carotid femoral pulse wave velocity (PWV) was estimated by means of an automated method (Complior SP). Moreover, in all subjects venous blood samples were drawn for estimation of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6).
Results: HIV patients compared to controls had lower diastolic brachial blood pressure (BP) (by 4.8 mmHg, $p = 0.003$), higher brachial pulse pressure (by 3.7 mmHg, $p = 0.045$), decreased aortic systolic and diastolic BP (by 4.6 mmHg, $p = 0.049$, and by 5.7 mmHg, $p = 0.017$), while there was no difference in aortic pulse pressure and PWV values. In addition, heart rate was increased (by 6.4 bpm, $p = 0.012$) and AIx along with AP were decreased (by 6.4%, $p = 0.048$ and by 3.3 mmHg, $p = 0.010$) in subjects with HIV infection. HIV-infected patients compared with controls had significantly increased values of log hs-CRP ($p = 0.007$) and log IL-6 ($p = 0.048$).
Conclusions: HIV infected naive patients without AIDS compared with controls are characterised by similar values of large artery stiffness and decreased wave reflections. Peripheral vasodilatation is suggested to be the predominant mechanism, induced probably by the chronic subclinical inflammation as reflected by increased inflammatory markers in this setting. These findings provide further insight into the pathogenesis of HIV-related cardiovascular risk.

P1281 Parvovirus B19 and Erythrovirus type 2 and 3 infections are infrequent in HIV-infected patients with CD4 <500 cells/mm^3 and chronic anaemia

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Background: Eryhthroviruses, Parvovirus B19 (PVB19) and the newly described Erythrovirus genotype 2 and 3, mainly target human erythroid progenitors. The pattern of clinical disease due to PVB19 infection is strongly influenced by the haematologic and immunologic status of the host. In immunosuppressed patients, a reactivation of PVB19 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/mm^3 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/mm^3 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/mm^3, 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 ultrasensitive PCR technique

P1282 Non-AIDS defining malignancies in the era of combined antiretroviral therapy

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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'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 connected 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 myelogenic 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 adnocarcinoma (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 mimick and/or overlap infectious complications. Further epidemiological and clinical investigation is strongly warranted to increase the awareness of this emerging phenomenon.

P1283 Long-term effects of occult hepatitis B virus infection in human immunodeficiency virus infected patients

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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 surface 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 incident 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/mm^3 and 146.6±21.9 cell/mm^3 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.

P1284 Non-vaccine HPV types are frequent in HIV-positive subjects

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Introduction: We examined anal HPV infection in a cohort of HIV infected patients and correlated cytological findings with molecular findings and immunological parameters.
Methods: Prospective follow-up with Thin prep anal smear testing is performed in all patients of an tertiary care centre HIV clinic. Clinical arrays are used to identify HPV infection. Univariate and multivariate analysis was used to identify associations between HPV detection and demographics, cytological changes and immunological parameters.
Results: 105 pts [92.3% male, median age 38 yrs (IQR: 30.3–44.8)] with a median duration of HIV infection of 3 yrs (IQR: 1–7.8 yrs) have been examined. Mean value of current CD4 was 419/mm3 (IQR: 284.5–593.8) and of current HIV viral load was 364.5 c/mL (IQR: non detected-9880 c/mL, 48/105 <50 c/mL). Cytological changes were detected in 61.5% of the population. AIN1, AIN2, and AIN3 were diagnosed in 3.7%, 1.2% and 2.5% of the population respectively. HPV was detected with clinical arrays in 92.4% of the population (69.2% low risk types, 83.3% high risk types, 59.1% mixed low and high risk types). Most frequent types detected were HPV types 6 (40%), 53 (39%), 51 (28.5%), 11 (25.7%), 66 (22.8%), 70 (21%), eae 18 (19%). Diagnosis of a cytological lesion were associated with HPV detection (p = 0.01). HPV positivity was associated with decreasing age (p = 0.02), n of sexual partners (p = 0.045), and lower CD4 counts (p = 0.02).
Conclusions: HPV infection was prevalent in this patient cohort. Non vaccine HPV types predominated. Immunological correlates of HIV infection appear to be important in the evolution of anal cytological changes. Prospective follow-up of HIV pts with HPV is necessary for the prevention of perianal malignancies.

P1285 The association between changes in depression and adherence to highly active antiretroviral therapy among adult HIV-infected patients in Thailand

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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.

P1286 Non-invasive assessment of liver fibrosis by measurement of stiffness in HIV-infected patients. The significance of the metabolic syndrome

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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 stiffness of >7.4 kPa was considered to define presence of 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, mean BMI 24.5 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 three 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 HBV or HCV co-infection. Therefore, such patients may be benefit from therapeutic intervention aiming to the management of metabolic syndrome parameters.

P1287 Erectile dysfunction in HIV+ male patients

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Objectives: To assess the prevalence of ED and to identify potential risk factors in a HIV(+) male population.
Methods: For a 3 month period, the "International Index for Erectile Function" questionnaire (IIEF) was distributed to HIV+ male pts attending the outpatient clinic for regular follow-up. Pts with recent HAART initiation (<6 mos), recent hospital admission (<1 mo) and recent HIV diagnosis (<1 y) were excluded. For each pt, ED was assessed by the IIEF score. Demographic information, clinical data (HAART history, comorbidities) and lab data were recorded (CD4 count, viral load expressed as logVL, thyroid function tests, FSH/LH, prolactin and testosterone serum levels, lipid profile). Mann-Whitney and Kruskal-WallisH tests were applied for quantitative data and chi-square test for qualitative data. Variables with P value <0.1 were entered in a multivariate logistic regression stepwise model, calculating odds ratios(OR), 95% confidence intervals(95%CI). P values $\leqslant$0.05 were considered as statistically significant. Informed consent was obtained from each patient enrolled.
Results: Fifty pts were originally enrolled, and 47 provided a properly filled questionnaire. Mean pt age($\pm$SD) was 39$\pm$11.2 ys with mean CD4 count($\pm$SD) 518$\pm$13.7 cells/mm^3 and logVL($\pm$SD) 2.7$\pm$1.2. Thirteen pts were not actively on HAART (23.6%). Half of the pts had undetectable viral load (logVL $\leqslant$1.7). No pt had a history of hypertension, coronary heart disease or psychiatric illness. Smoking was reported in 30%. In 21/47 pts (44.7%) ED of any degree was revealed. Prevalence of ED was 21% for pts <35 ys, 55% for those 36–50 ys and 75% for pts >50 ys. Risk factors identified by univariate analysis were age (P = 0.009), nadir CD4 count (P = 0.017), history of AIDS (P = 0.023), more than one HAART regimens (P = 0.023) and actively receiving a PI-based regimen (P = 0.021). No difference was identified in the other haematological and biochemical parameters tested. From the multivariate analysis, age (for pts <35 ys, OR 0.104, 95%CI 0.020–0.537, P = 0.007) and nadir CD4 count (for <200 cells/mm^3 OR 22.121, 95%CI 1.212–403.728, P = 0.037) displayed significant association with the presence of ED.
Conclusions: ED is prevalent among HIV+ male pts, presenting at an earlier age than the general population. Pts with lower nadir CD4 counts and age >35 years present more frequently with ED. A larger number of pts will help elucidate other possible risk factors associated with the presence of ED in HIV+ pts.

P1288 Soluble urokinase-type plasminogen activator receptor levels predict mortality in HIV+ patients

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Background: Urokinase-type plasminogen activator (u-PA) 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 to 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= 0.451, 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.

P1289 Response to anti-retroviral treatment at an AIDS centre, Jerusalem: comparison between Ethiopian and non-Ethiopian HIV+ patients

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Objectives: Until 2007, 4999 HIV patients were reported by the Israel Ministry of Health, 2167 of whom (43.3%) originated from countries endemic to HIV, mainly Ethiopia. In this study we aimed to retrospectively compare disease, treatment and resistance patterns among Ethiopian immigrants vs. non-Ethiopian patients treated at a regional AIDS centre.

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 Ethiopian 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(408.3±190.1 cells/ml) was lower than that in non-Ethiopians (616.8±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 mutations, the 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 treatment, 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

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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(r)+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/mm^3 [interquartile range IQR: 114–318] and 4.3 log10 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 $\geqslant$ 100 cells/mm^3 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.

P1291 HIV/AIDS epidemics development of pregnant woman in Ukraine

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Ukraine appears to be at the centre of the HIV-epidemic in Europe, with twice as many newly registered HIV cases as in Western Europe and 14 times as many as in Central Europe. In 2008 approximately 1.46% of the total adult population was estimated to be HIV positive – 440,000. In Ukraine, the rate of HIV-infected dissemination among pregnant women is one of the highest in Europe. This parameter equals to 0.45% of all pregnant women in 2008, while it was only 0.002% in 1995. The increase in the number of the HIV-infected women is of additional concern because of the risk of vertical transmission from mothers to their children. In Ukraine 86% of the patients of reproductive age who are being treated are women. It is worth mentioning that daily 10 children are born to HIV-infected mothers in the south of Ukraine. Early diagnosis, treatment and behaviour counseling is crucial to avoid transmission and limit the damaging effects of the disease.
The studies performed by Ukraine explorers show us that about 12%–38% HIV-infected mothers became informed on their positive status only at the birth-houses. Odesa region is at the first place among other regions of Ukraine as to the level of HIV/AIDS infection cases and at the third as to the infected pregnant women In the case of the serum-positive response, the infected woman needs the information on the probability of infecting the child during birth and how to avoid transmission after delivery. In addition, the provision of such information in time could decrease the number of HIV-infected who obtain anti-retroviral therapy which will help diminish vertical HIV transfer from mother to child. Ukraine have entered the seven countries' circle and are attempting to provide access to antiretroviral therapy for HIV-positive pregnant women. This effort is beginning to reduce the number of HIV-positive newborns. During last 10 years HIV infected pregnant women number increased by 17% (from 28% to 45%).
Permanently increases the number of children born by HIV-infected mothers. However, the level of the anti-retroviral therapy provided to HIV-positive pregnant women was equal only to 11%. Anti-retroviral therapy applied timely allowed to diminish the newborns HIV infection from 30% to 9%. As the result, more than 1295 previously infected children were qualified as healthy.

P1292 Ortotopic liver transplantation in HIV-infected patients. Outcome of a single-centre cohort of 21 patients

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Objectives: To present the outcomes of the HIV-infected patients treated with orthotopic liver transplantation (OLT) in our institution.
Methods: HIV-infected patients treated with OLT in our institution were prospectively studied. Our inclusion criteria for OLT were as follows: (1) criteria for liver disease was the same to non-HIV patients; (2) patients with previous HIV C events were not accepted except some OIs (TB, Can, PCP); (3) Pre-OLT CD4 count >100 cells/mm^3; (4) RNA HIV-1 viral load BDL on antiretroviral therapy (ART) or, if detectable, post-OLT suppression predicted; (5) No heroin or cocaine use for >2 years and no alcohol use for >6 months.
Results: From September 03 to December 08, 22 OLT with deceased donors were performed in 21 HIV-infected patients. Fifteen were male (71.4%) with a mean age of 44 years (range 33–60). HIV risk factor was former i.v. drug abuse in 17 patients, transfusion in 2, sexual in 1 and haemophilia in 1. The aetiology of liver disease was HCV infection in 14 patients, HCV/HBV coinfection in 6 and cryptogenic in 1. Nine patients were HCV genotype 1/4 and 7 were genotype 2/3. Eight patients had a hepatocellular carcinoma. Mean MELD score was 12 (r 6–22). Pre-OLT antiretroviral treatment (ART) was based on efavirenz in 6 patients, on PI in 5 patients and on NRTIs in 9 cases. One patient had no ART.

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/mm^3 (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.

P1293 Genetic profiles of HIV-1 populations in patients on failing abacavir/lamivudine/zidovudine

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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 timepoint.

Results: Drug resistant mutations were detected in five patients post treatment failure. Phylogenetic analysis showed that viral sequence at different timepoint 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.

P1294 Discordant genotypic interpretation and phenotypic role of protease mutations in HIV-1 subtypes B and G

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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 Egas 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 patients were phenotyped for susceptibility to all available PI through VIRCO's Antivirogram®, and compared to a similar dataset of subtype B patients. Mutation I54V/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.

Antifungal resistance

P1295 Efficacy of voriconazole against three clinical *Aspergillus fumigatus* isolates with mutations in the cyp51A gene

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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 long 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.

P1296 Development of azole resistance in a clinical *A. fumigatus* isolate with no mutations in the cyp51A gene

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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 gram. 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) 12 mg/kg, posaconazole (POS) 20 mg/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 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 monotherapy 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+voriconazole 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.

P1297 Is *Aspergillus nidulans* susceptible to all antifungal agents? In vitro activity of an updated panel of antifungal agents against 63 clinical isolates

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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 (0.5/2/0.61/0.062–2), posaconazole (0.5/1/0.54/0.25–1) and isavuconazole (0.25/1/0.46/0.062–1). Amphotericin B showed the lowest activity (52.3% of strains with MICs $\geqslant 4$ μg/mL) ($P < 0.001$). In contrast, terbinafine showed good activity (98.4% of strains with MICs $\leqslant 0.25$ 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 $\leqslant 1$ μg/mL) ($P = 0.05$), followed by posaconazole, and voriconazole. Itraconazole showed the lowest activity (20.7% of strains MICs $\geqslant 2$ 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–voriconazole (PC=0.864), 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.

P1298 In vitro antifungal activity of isavuconazole against 345 mucorales isolates, collected at eight study centres worldwide

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Objective: Although mucormycosis (infections due to *Absidia*, *Mucor*, *Rhizomucor* and *Rhizopus* spp.) are relatively uncommon, they are associated with high mortality and current treatment options are limited. Isavuconazole (ISA) is a novel broad-spectrum azole with excellent bioavailability, currently in late-stage clinical development for the treatment of invasive aspergillosis and candidiasis. The objective of this study was to collate data from independent in-vitro studies evaluating the antifungal activity of ISA against a large and diverse collection of mucorales clinical isolates ($n = 345$), collected and tested at eight study centres in Europe, Mexico and North America.
Methods: Each study centre undertook MIC susceptibility testing of isolates collected at their institution, according to EUCAST (1 centre) or CLSI (7 centres) guidelines. The 345 isolates comprised: 80 *Absidia* spp. (49 *A. corymbifera*; 31 unspecified), 18 *Cunninghamella* spp. (3 *C. bertholletiae*; 15 unspecified), 77 *Mucor* spp. (19 *M. circinelloides*; 2 *M. ramosissimus*; 1 *M. rouxianus*; 55 unspecified), 29 *Rhizomucor* spp. (11 *R. pusillus*; 18 unspecified), 139 *Rhizopus* spp. (28 *R. arrhizus*; 44 *R. microsporus*; 15 *R. oryzae*; 52 unspecified) and 2 *Syncephalastrum* spp. Additional tests (E-test, hyphal growth inhibition) were conducted at some laboratories.
Results: Across all study centres and following 24 h incubation (48 h by EUCAST methodology), ISA exhibited MIC50 values of 1 to 4 mg/L and MIC90 values of 4 to 16 mg/L against the five genera. Regardless of genera tested, there were noticeable differences in the MIC distributions obtained from each study centre. These differences could not be ascribed to differences in inoculum and/or endpoint determinations. MIC distributions using EUCAST methodology fell within the MIC ranges for study centres using CLSI methodology. All MICs were higher following 48 h incubation. MICs of ISA against hyphae were similar to those obtained against the standard conidial suspension. The E-test tended to generate higher MICs.
Conclusions: Laboratory variation in the ISA MICs obtained was found. ISA possessed at least moderate in vitro antifungal activity against Mucorales. If ISA is confirmed to yield high levels in serum and relevant tissues it could have a role in the prevention and treatment of mucormycosis.

P1299 In vitro combination of amphotericin B or posaconazole with cyclosporine A against zygomycetes

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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 *Mycocladus 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.

Amphotericin B or posaconazole Etest strips were applied on the agar surface and MIC of antifungals were determined after 24 h of incubation at 35ºC.

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.

Isolate	MIC (mg/1) of the drugs alone		Concentration (mg/l) of CyA in plates	MIC (mg/l) of the drugs in combination with CyA	
	AMB	PSZ		AMB	PSZ
R. oryzae IP 1443.83	ND	1	16	ND	1
R. oryzae IP 4.77	>32	>32	0.5	>32	2
R. oryzae CNR 03.918	>32	2	16	0.09	0.5
R. microsporus AZN 1185	0.5	>32	0.5	0.5	>32
R. microsporus IP 676.72	2	2	0.25	1.5	2
M. circinnelloides CNR 03.154	0.5	3	2	0.06	1
M. circinnelloides CNR 03.371	0.38	>32	2	0.03	16
Rh. pusillus IP 3.77	0.38	0.19	0.125	0.25	0.125
A. corymbifera IP 1280.81	2	0.25	16	0.125	0.03
A. corymbifera IP 1279.81	ND	0.5	16	ND	0.06

CyA: cyclosporine A; AMB: amphotericin B; PSZ: posaconazole. ND: Not done.

P1300 Molecular mechanisms of azole resistance in *Candida glabrata* clinical isolates from Slovakia

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Objectives: The aim of this study was to investigate the molecular mechanisms involved in a decreased susceptibility to azole antifungals in unmatched *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 to azole antifungals were investigated in ten unmatched *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 CgPdr1p in one and five clinical isolates, respectively. Sequencing of the CgERG11 gene encoding 14C-lanosterol demethylase and analysis of its expression identified the E502V amino acid substitution in five of ten isolates and showed no upregulation of this gene in any clinical isolates. No significant association was observed among the drug susceptibility profiles, mechanisms of azole resistance and the cell surface hydrophobicity correlating with the ability of isolates to form biofilm.

Conclusion: The results demonstrate that decreased susceptibilities of *C. glabrata* clinical isolates to azole antifungals are associated with 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 flucytosine and amphotericin B in clinical yeast isolates

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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 flucytosine (FC) and amphotericin B (AM), 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 (10^5 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 (SPs).

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%), Magnusiomyces (8/0.6%), Filobasidiella (2/0.2%), Issatchenkia (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 (24 h vs. 48 h): 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*, Issatchenkia, 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 24 h 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

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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 with reduced susceptibility 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 10^4 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 $>3\log_{10}$.
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 mg/L, 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) mg/L, 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*

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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 at the 6 and 8 hr 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 4 hr intervals, 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 Cp. 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

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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 35ºC 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 CS. In terms of resistance 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*

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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 aetiologic 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), itraconazole (ITC), voriconazole (VRC), ravuconazole (RVC), posaconazole (POS), caspofungin (CAS), micafungin (MCF) and anidulanfungin (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 $\leqslant$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 ITC, VRC, RVC and POS (MICs >1 mg/L) were detected in 18.5%, 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.

P1306 Antifungal susceptibility of 205 *Candida* yeast isolated during systemic candidiasis. Comparison of the Vitek 2 system with E-Test and CLSI broth microdilution methods

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Objectives: The objective of this study was to evaluate a new automatised 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 amphotericin B, fluconazole and voriconazole.
Methods: The CLSI and the Etests MICs were determined at 24 and 48 hours of incubation at 35ºC. The amphotericin 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 only 1 isolate was 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 of 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 amphotericin B, a MIC $\leqslant 1$ was globally found for 201 (VK2), 190 (CLSI), and 202 (etest) isolates. One *Candida glabrata* isolate had a MIC $\geqslant 2$ in the three methods.
Conclusion: The AST-YS01 Vitek 2 cards system (Biomerieux) is a reliable and practical automatised antifungal susceptibility test.

P1307 Comparison of the Vitek AST-YS01 card with CLSI broth microdilution reference method for testing fluconazole and voriconazole against *C. neoformans*

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Objective: The purpose of this study was to compare the results obtained by the reference microdilution method (MDM) with those obtained with the comercial method AST-YS01card (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 nº2 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$\geqslant$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 cathegorical agreement between both methods for FZ is summarised in table 1. YS01 was not able to identify the *C. neoformans* isolates resistant to FZ (2/4, 50%).

Table 1

MIC (category), no. (%)		CA (%)	Errors, no. (%)		
S	R		Very major	Major	Minor
Vitek: 86 (96.6) CLSI: 85 (95.5)	Vitek: 3 (3.3) CLSI: 4 (4.4)	96.6	2 (2.24)	1 (1.12)	–

Conclusions:
1. These data suggest the potential value of YS01for determining the MICs of FZ to susceptible *C. neoformans* isolates.
2. YST01 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.

P1308 Does minimum inhibitory concentration of echinocandins impact clinical outcome? First European case of breakthrough *Candida glabrata* candidaemia successfully treated with micafungin

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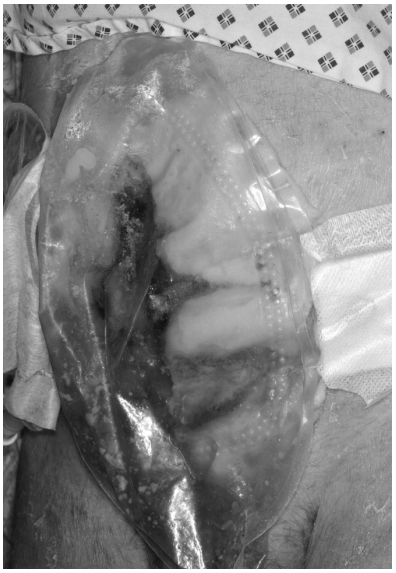
Introduction: The echinocandins are the first new class of antifungals that target the fungal cell wall by blocking β-1.3-d-glucan synthase. Literature reports an uncertain correlation between clinical failure and elevated MIC values for the echinocandins. We present the 1st case in Europe successfully treated with micafungin for repeated breakthrough *Candida glabrata* candidaemia during caspofungin therapy.
Case study: A 16-year old male (weight 50 kg) was admitted in July 2008 with 4-day history of lower abdominal pain. An emergency laparotomy revealed large perforation of mid-sigmoid colon and extensive faecal contamination of peritoneum. He underwent Hartmann's procedure and abdominal closure with drainage. Patient developed wound infection and CT-diagnosed pelvic collection. Following dehiscence of abdominal wound he subsequently developed an enterocutaneous fistula. This is draining several litres of effluent a day through a closed sealed transparent dressing into a large bag. He was treated with total parenteral nutrition his transfer to specialist hospital in October 2008.
During 3-months of hospital stay the patient had 5-central line, 2-peripherally inserted central catheter [PICC] and 2-Hickman line changes following repeated candidaemia. The patient had 9-candidaemias with *C. tropicalis* [between Jul 30 and Aug 20] treated Fluconazole (400 mg/d) followed by caspofungin (50 mg/d). This was followed by 7-candidaemias with *C. glabrata* [between Sept 19 and Oct 4]while he was treated with Caspofungin [50 mg/d]. Patient received his last hickman line under cover of caspofungin and voriconazole in combination and then changed to micafungin (100 mg/d). Taurolidine – 4% citrate line lock used in the new hickman line. Micafungin was used for 2-weeks & taurolidine line lock for 12-days and then stopped. The patient [hickman line – now 45-days] is on TPN without further infection episodes.

The 7-isolates of *C. glabrata* were tested in-house [Fluconazole 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 casofungin in a susceptible *C. glabrata* strain is of concern. Micafungin, newest of echinocandins, has lower MICs against *C. glabrata* and offered clinical success.



P1309 Caspofungin non-susceptible *Candida* spp. isolates in cancer patients

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Objectives: Caspofungin (CAS) has emerged as one of the main choices for primary treatment of candidiasis. Despite extensive use, resistance (R) to CAS and cross resistance among the other echinocandins is considered rare in the clinic.
Materials and Methods: We reviewed the in vitro susceptibilities (CLSI method) of 650 *Candida* species associated with candidaemia or non-candemic 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 CAS in the previous 3 months. Neutropenia, and prior corticosteroid treatment were seen in 5 (71%) and 6 (86%) pts respectively. Six of 7 (86%) of CAS-R Candidiasis episodes were breakthrough infection to systemic antifungals. Five (71%) of CAS-R-*Candida* isolates were isolated from blood and 1 each from pelvic abscess the urine (in a pt with nephrolithiasis). *Candida tropicalis* in 3 (43%) was the most common *Candida* spp isolated. One isolate (*C. glabrata*) had multi-resistance to azoles, amphotericin B and echinocandins. All 7 pts responded to change of antifungal therapy (catheter was removed in all 5 candidemic pts).
Conclusion: CAS-R candidiasis appears rare, even in a pt population where there is extensive CAS use. Prospective surveillance studies are needed.

P1310 Pharmacokinetics of caspofungin in patients with candidaemia

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Objectives: The knowledge of pharmacokinetic/pharmacodynamic (PK/PD) properties of antifungals and the role of therapeutic drug monitoring (TDM) in the clinical setting are still limited. Caspofungin is an antifungal belonging to the class of echinocandins and is approved as first line treatment for invasive candidiasis. Caspofungin displays a concentration-dependent activity and the AUC/MIC has been demonstrated to be the PD parameter linked with efficacy in the murine model. Aim of our study was to measure caspofungin plasmatic parameters in patients with candidaemia.
Methods: We studied non-obese adult patients with candidaemia treated with iv caspofungin at the recommended dosage. Plasma samples for PK measurements were collected at the steady state condition, immediately before caspofungin infusion and 1, 2, 5, 9 and 24 hours after the daily dose. Caspofungin plasma concentrations were determined using a fully validated HPLC-UV method. Pharmacokinetics parameters were obtained using WinNonlin software. Cmax/MIC and AUC/MIC were calculated considering the highest MIC reported for susceptible strains.
Results: Twelve patients (9 males, 3 females) were evaluated. Five 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+SD Cmax, Cmin and AUC were respectively 8.36+2.18 mg/L, 1.98+0.87 mg/L and 80.45+28.84 h*mg/L. 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.

P1311 *Pneumocystis jirovecii* dihydropteroate synthase gene mutations and sulfa resistance

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Background: Sulfa drugs, trimethoprim-sulfamethoxazole and dapsone, are mainstays for prophylaxis and treatment of *Pneumocystis* pneumonia (PcP), a life-threatening disease in immunosuppressed patients. The inability to culture Pneumocystis has led to develop molecular techniques based on identification of punctual mutations on the Dihidropteroate Synthase gene (DHPS) that cause sulfa resistance in other microorganisms. A key issue is whether the emergence of DHPS mutations is result of transmission between patients or arises from selection by the pressure of sulfa drugs, two possibilities are not mutually exclusive. The role of Pneumocystis colonised subjects in transmission of DHPS mutations still unknown. The aim is to provide epidemiological data of *P. jirovecii* DHPS mutations among PcP patients and immunocompetent colonised subjects.
Methods: The study included 47 PcP patients and 75 Pneumocystis colonised subjects during 2001–2007 identified by nested PCR at mtLSUrRNA gene. DHPS mutations were studied by Restriction Fragment Length Polymorphism using Acc I and Hae III at nucleotide positions 165 and 171 respectively.
Results: The analysis showed a 19.7% prevalence of DHPS gene mutations in the overall population. All possible polymorphisms

described were identified. There were not difference between the frequency of DHPS mutations in PcP patients and colonised subjects (23.4% vs 17.3%; p = 0.75). A trend towards decreased frequency was observed during this period (31.3% of DHPS-mutations during 2001 to 11.6% at 2007).

Conclusions: Similar DHPS pattern was observed in PcP patients and immunocompetent colonised subjects suggests that both group could share a common transmission cycles of mutated strains, and arise question about the role that colonised subjects could represent as reservoir for DHPS mutations with ability to transmit them to immunocompromised hosts susceptible to PCP.

Molecular bacteriology – part 2

P1312 A new real-time PCR assay for the direct detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*

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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 in 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 resulting to 100% sensitivity and 98.7% specificity compared to Roche techniques. These 3 Bio-Rad FP samples contained *N. meningitidis* and came from 3 male patients. IAF consulting population is mainly constituted of homosexually active men with cross-contaminations.

MG: 12 samples were found positive with the Bio-Rad test, among them only 8 were concordant with the reference in-house test. The 4 discordant Bio-Rad extracts were re-evaluated with a third PCR technique, confirming their positivity.

Among the 12 positive samples, 6 were co-infected with either CT or NG and 6 were CT or NG negative including 3 samples from patients with clinical signs (uretritis and cervicitis).

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.

P1313 Evaluation of a commercial assay for fast and efficient detection of *Chlamydia trachomatis*-DNA in clinical specimens with a rapid PCR-based dipstick assay

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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 in to a tube and centrifuged. The supernatant was discarded and the pellet resuspended in 100 µl of lysis reagent. After a 5 min heating step in boiling water 100 µl of neutralistion 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 titer plate. The stick contains 3 lines for wild type detection, amplification control and run control and were evaluated after 10 min by eye.

Samples were analysed in parallel with DNA-isolates derived from an automated DNA isolation system (Chemagic MSM I, Chemagen, Baesweiler, Germany) and amplified with the Roche COBAS TaqMan 48.

Results: The GQ CT assay showed for urine specimens 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.

Conclusions: 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.

P1314 *Chlamydia trachomatis* detected in a Croatian national public health institute between 2004–2008

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Objectives: *Chlamydia trachomatis* is a pathogen of global public health significance. Its infection is one of the most important sexually transmited 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, ABI 7500, 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 preformed 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, 4/640 (0.6%) sperm specimens and 19/814 (2.3%) EPS. There was no inhibition 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.

P1315 A molecular assay detecting quinolone resistance in *Neisseria gonorrhoeae*: treatment with ciprofloxacin can still be an option

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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 susceptibility. Susceptibility was tested using disk diffusion according to the Dutch CRG guidelines (S: $\geqslant$36 mm; I: 35–30 mm; R: <30 mm). A total of 26 susceptible, 6 intermediate and 31 resistant isolates were evaluated. In case of discrepant results, E-testing was performed (S: MIC $\leqslant$0.06; I: MIC 0.12–0.5; R: MIC $\geqslant$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.

P1316 Metagenomic assessment of sputum microbiota from patients with chronic pulmonary diseases

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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 60ºC; with a urea-formamide denaturating gel gradient of 30–45%. Gels were visualised with ethidium bromide; common and unique bands were excised, re-amplified and sequenced in order to assign bacterial species identification. Similarities among the electrophoretic band patterns were analyzed using the Phoretrix 5.0 software® and dendrograms were constructed based on the Dice coefficient.
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* synxantha, Ochrobactrum anthropi, Rothia amarae, Rothia mucilaginosa, Phycicoccus dokdonensis, or Arthrobacter 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.

P1317 Use of novel electrochemical labels in a highly sensitive, multiplex detection method for use in a rapid point-of-care molecular assay

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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: A *Chlamydia trachomatis* specific PCR primer set was used to asymmetrically amplify target DNA extracted from Elementary Bodies (EB). A single-stranded DNA probe was synthesized with the electrochemical label linked to the 5′ base via the terminal phosphate group. This probe was hybridised to the *C. trachomatis* amplicon, 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.

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

Sample	Oxidation potential (mV)	Mean electrochemical measurement (nA)	Standard deviation	%CV
1000 *Ct*EB*	192	216.33	11.02	5.1
100 *Ct*EB*	198	109.47	25.17	23.0
10 *Ct*EB*	198	65.07	4.66	7.2
1 *Ct*EB*	201	54.97	25.29	46.0†
No DNA/EB Control	–	0	–	–
E. coli	–	0	–	–
Strep. agalactiae	–	0	–	–
Candida albicans	–	0	–	–
N. gonorrhoeae	–	0	–	–
E. faecalis	–	0	–	–
S. aureus	–	0	–	–
Lactobacillus sp.	–	0	–	–
T. vaginalis	–	0	–	–
Human	–	0	–	–

**Ct*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

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Objective: *Staphylococcus hominis* strains are one of the major staphylococcal species inhabitating the skin of humans. *Staphylococcus hominis* is subdivided in the subsp. *hominis* and *novobiosepticus*. *Staphylococcus hominis* subsp. *novobiosepticus* is a multiresistant pathogen occuring 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

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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

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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 regulaon 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 initiating 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

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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

specific. In the present study we evaluated such an assay, comparing its output to the method currently in use in the Clinical molecular diagnostic lab of our hospital.
Methods: Forty patient samples and 5 quality control samples were used for the evaluation of the MTB Q Alert Real-Time-PCR kit from Nanogen. All Patient samples had been routinely tested for *Mycobacterium tuberculosis* by the Amplified MTD Assay (Genprobe).
Results: All Real-time-PCR results obtained with the MTB Q Alert kit proved to be completely concordant with the Amplified MTD results. Also, all quality control samples tested were found to generate concordant results with the expected results provided by the external control panel manufacturer. However, 33% of the patient samples studied did not generate Real-time-PCR data due to PCR reaction inhibition attributable to the sample.
The Real-time-PCR assay was found to have a 100% sensitivity, specificity, Negative predictive value and positive predictive values, but seems to be useless in a significant number of patient samples due to inability of the extraction procedure to remove PCR inhibitors from the sample. Furthermore, the extremely labour intensive extraction procedure counterbalances the Real-time-PCR speed, drastically reducing its advantages in the clinical setting. In addition, the use of the relatively slow ABI 7300 platform further reduces the advantage of the Real-time-PCR in an assay that should be performed as soon as sample arrives to the lab.
Conclusion: In conclusion, the amplification assay proved to be very sensitive and specific, but its usefulness was severely damaged by the DNA extraction procedure. Future work on alternative preferably automated extraction procedures should be done to obtain maximal usefulness of the amplification procedure.

P1322 Development and application of a multiplex polymerase chain reaction assay for rapid differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis

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Objectives: The aim of this study was to develop a multiplex real-time PCR (M RT-PCR) assay to simultaneously detect *Brucella* spp and *M. 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.

P1323 Identification of *Nocardia* from respiratory specimens by using 16SrRNA gene sequencing in Zaragoza, Spain between 1998 and 2003

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Objectives: To determine the incidence of *Nocardia* sp. from respiratory specimens in the H.U. Miguel Servet between 1998 and 2003.
Identify *Nocardia* to the species level using 16SrRNA gene sequencing.
Methods: Respiratory samples were cultured in blood, chocolate, Sabouraud and BCYE agar and were incubate for 7 days at 37ºC. In this period of 5 years, 125 *Nocardia* strains were isolated from bronchoalveolar lavage fluids, bronchial secretion aspirates and sputa. The identification was performed on the basis of visualisation of colony coloration and morphology, Gram and modified acid-fast staining, adenine, casein, xanthine, hypoxanthine, tyrosine, Middlebrook and antimicrobial susceptibility patterns. Complete molecular identification was performed in the French Observatoire for Nocardiosis based on 16S rRNA gene sequencing.
Results: Between 1995 and 2003, were diagnosed 125 cases of respiratory isolates being 48 females (38%) and 77 males (62%). The following species were isolated: *Nocardia abscessus* (n = 45), *Nocardia farcinica* (n = 24), *Nocardia cyriacigeorgica* (n = 14), *Nocardia transvalensis* (n = 4), *Nocardia veterana* (n = 5), *Nocardia nova* (n = 3), *Nocardia pneumoniae* (n = 5), *Nocardia asteroides* (n = 2), *Nocardia carnea* (n = 8), *Nocardia cerradoensis* (n = 1), *Nocardia flavorosea* (n = 1), *Nocardia ignorata* (n = 1), *Nocardia otitidiscaviarum* (n = 1), *Nocardia testacea* (n = 1) and *Nocardia* spp (n = 10).
Conclusions: The present study shows the first results of nocardial incidence in Zaragoza and exposes the predominance of *Nocardia abscessus* (36%) and *Nocardia farcinica* (19%). The development of methods like 16SrRNA gene sequencing will facilitate rapid diagnosis, epidemiological studies and prompt the initiation of the appropriate chemotherapy. The incidence of *Nocardia* spp. in our hospital is remarkable so when physician have one suspicion about this infection in patients with risk factors, should informed to microbiologists to include specific media cultures and be incubated for a long period of time.

P1324 Genetic analysis of the cap locus of *Haemophilus influenzae* serotype E

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Objectives: Although immunisation of infants with conjugate vaccines against *H. influenzae* serotype b (Hib) has dramatically decreased the incidence of invasive Hib disease in developed countries, the potential for emergence of non-vaccine preventable strains has been suggested. Invasive disease caused by *H. influenzae* serotype e (Hie) strains has been recently observed in Italy, suggesting the importance of further molecular investigations on Hie genetic locus for the capsule biosynthetic genes (cap locus). The sequence of the entire Hie cap locus has not been previously described. In the present study, the Hie cap locus was characterised.
Methods: The location of the Hie cap locus within the chromosome was investigated in 11 invasive Hie isolates by PCR amplification and sequencing of the DNA regions flanking the ends of the locus. The cap locus from the invasive strain Hie 274 was sequenced. To encompass the entire cap locus, overlapping amplicons, ranging from 1500 bp to 9 kb, were generated by PCR. Each amplicon was then subcloned into pCR4-TOPO and the insert from each plasmid was sequenced. In all isolates, the copy number of the Hie cap locus was determined by Southern blot analysis.

Results: In each isolate, the chromosomal location of the Hie cap locus was identical to that previously described for *H. influenzae* serotype f strain 7000222 and associated with the same flanking genes (sodC at the 5prime; end and HI1637 at the 3′ 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 I (majority of serotypes a, b strains and all serotypes c, d, and e 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

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Objectives: *Vibrio cholerae* is a natural inhabitant of the aquatic environments. 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 UPGMA 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 stucture. 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*

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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 two *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 Gln. 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

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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* (CJ), Shiga toxin-producing *Escherichia coli* (STEC), 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 CJ, 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, CJ, 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

0.2% for SH. In comparison with conventional culture, the detection of bacterial enteric pathogens, increased from 9.6% to 15.2% using the molecular approach.

Conclusion:

1. In comparison with conventional culture, using a molecular screening approach improved the detection of bacterial enteric pathogens significantly.
2. Pre-screening with PCR increased the proportion of *Campylobacter* culture positive stools (7.0% versus 5.8%) significantly.
3. This new molecular strategy in screening stool specimens for gastro-intestinal pathogens has a potential to expand the mPCR enteric panel with viral, parasitic and other bacterial targets.

P1328 Development and evaluation of internal amplification controls for real-time duplex PCR to detect *Campylobacter coli* and *C. jejuni*

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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 IAC's 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 and/or jejuni) present at c. 10^8, 10^6, 10^4 and 10^2 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 this was reversed by dilution of the competitive IAC to 10–7. The signal from the 16S IAC was lost in the presence of strong positive signal for either *C. coli* or *C. jejuni*, whereas the signal from the fish virus IAC was rarely affected by positive signals from *C. coli* and *C. jejuni* (Figure 1). Faecal supernatants inhibited PCR reactions down to dilutions of ~1/16 to 1/128 and both the competitive and non-competitive components of the PCR reactions were more sensitive to inhibition than the *C. coli* or *C. jejuni* components of the PCR reactions.

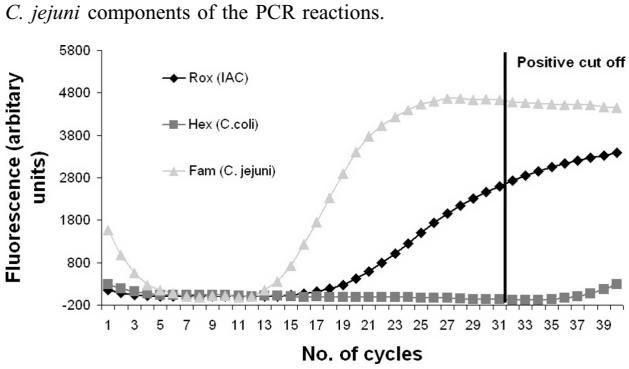

Figure 1. Fluorescence readings for a chicken faecal sample positive for IAC and *Campylobacter jejuni*.

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.

P1329 Whole genome sequencing of pathogenic and non-pathogenic treponemes: comparison of syphilis and yaws strains with *Treponema paraluiscuniculi*

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Objectives: *Treponema pallidum* subsp. *pallidum* (TPA) is the causative agent of syphilis, *T. pallidum* ssp. *pertenue* (TPE) causes endemic treponematosis yaws, and *T. paraluiscuniculi* is not infectious to humans but causes venereal spirochetosis in rabbits. TPA and TPE 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 frameshifts 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*.

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P1330 Study on the prevalence of tick-borne pathogens in the Lublin region (eastern Poland)

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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 (females, males and nymphs) collected from forest areas of six provinces 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 (Wlodawa district) were examined individually for TBEV by nested RT-PCR method according to Schrader and Süss 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 Radzyn Podlaski district was estimated as 4.2% and in the total area of the Lublin region was

estimated as 1.8%. The total infection rate of TBEV in Dermacentor reticulatus ticks from the Poleski National Park localities (Wlodawa district) amounted 1.4%.

Conclusions:

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.

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P1331 Towards a comprehensive proteome mapping of the opportunistic pathogen *Cronobacter turicensis* 3032

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Members of the genus *Cronobacter* 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 *Cronobacter 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 hydrolysing enzymes were found, suggesting that *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

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Objectives: Metalloproteases, particularly mirabilysin (ZapA), are considered important virulence factors of *Proteus mirabilis*. Mirabilysin 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 mirabilysin.

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 mirabilysin 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 mirabilysin (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 mirabilysin exhibited the typical N-terminus ZnMc serralysin domain, characterised by an α-β structure with a HExGHxxGLxH motif, and the C-terminus peptidase M10 domain formed by nine β-rolls with calcium binding G-rich nonapeptides.

P1333 *Actinobaculum schaalii*, a common cause of urine tract infections?

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Objectives: *Actinobaculum schaalii* is a slow growing, CO2 demanding, trimethoprim and ciprofloxacin resistent 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 patogen 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 37ºC 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 then 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 common patogenic bacteria present in the sample.

Conclusion: Real-time PCR is a fast and reliable method to identify *A. schaalii*. *A. schaalii* may be an important co-patogen in many urine tract infections. Patients suffering 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

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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 20 U/ml culture medium was reached in Batch fermentation with immobilised cells. In contrast with corresponding enzyme yield obtained with free cells, significant increases (2.2 fold) in 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 beads, enzyme yield decreased at high initial cell density.

P1335 Bacterial DNA in Egyptian patients with cirrhosis and culture-negative non-neutrocytic ascites: a marker of bacterial translocation and a prognostic indicator

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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* aereus 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.

P1336 Direct detection of *Bordetella pertussis* and *B. parapertussis* in clinical specimens by a rapid molecular dipstick assay

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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* in clinical specimens. The PCR-dipstick contains 3 lines, one for wild type detection, amplification control and run control, and is evaluated after 10 min by eye.
Methods: The lower detection limit of the assay was determined by serial dilutions of isolates of *Bordetella pertussis* and *B. parapertussis*. Analytical specificity was calculated with 35 "non-*Bordetella*"-isolates of different culture collections (ATTC, 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 (BioMérieux, Nürtingen, 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 clinial 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 is 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

P1337 Limitations of tpi and bg sub-genotypings for characterisation of human *Giardia duodenalis* isolates

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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 2 gene loci. High intra-assemblage genetic variability determined many subgenotypes with uncomplete 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 A3, 5/7 to genotype B3, 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.

P1338 Multilocus sequence typing of *Campylobacter jejuni* isolated from bovines, poultry and patients in Finland

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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://pubmlst.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.

P1339 Molecular characterisation of epidemic isolates of *Vibrio cholerae* O1 recovered from an outbreak occurred in different parts of Iran in 2005

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Objective: Aim of this study was to evaluate AP-PCR for investigation of clonal relatedness among the strains of *V. cholerae* recovered from an outbreak occurred in different parts of Iran in 2005.
Material and Methods: The study was conducted during the cholera outbreak occurred in some of provinces in Iran in summer 2005. 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

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Objectives: In Finland, microbiological laboratories send the *Salmonella* strains isolated from humans to the *Salmonella* 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 international 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)

[1] Lindstedt, B.-A., Vardund, T., Aas, L., Kapperud, G. 2004. J Microbiol Methods 59, 163–172.

P1341 Search for surface-anchored and strain-variable *Streptococcus agalactiae* proteins in clinical *Streptococcus pyogenes* isolates

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Objectives: Group A streptococci; (GAS) are among the most frequent causes of bacterial infections in humans. Group B streptococci: (GBS)

are a less frequent cause of infections in humans but still are important pathogens. Serotype determination of GBS is based on the capsular polysaccarides and serosubtype determination on a varity of surface-anchored an strain-variable proteins, including C beta (bca) C alpha (bac), Alp1 (alp1), Alp2 (alp2), Alp3 (alp3), Alp4 (alp4), R4(Rib) (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 possesion 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.

P1342 Pili are a clonal property of *Streptococcus agalactiae*

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Objectives: *Streptococcus agalactiae* (GBS) isolates express protective antigens recognized as major components of pilus-like structures. Pili were described in three distinct variants (PI-1, PI-2a and PI-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 multilocus 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 pili-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 PI-1+PI-2a and PI-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 pilus type indicate that pili are a clonal property of GBS.

P1343 The prevalence of pneumococcal pilus among invasive isolates of *Streptococcus pneumoniae* serotypes covered by 7-valent pneumococcal conjugate vaccine

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Objectives: The effective spread of certain invasive clones has not been fully elucidated yet. Apart from the capsule a number of virulence factors could have influenced the success of particular clones. One of the lately identified virulence factor is pneumococcal pilus encoded by rlrA pathogenicity islet. The pilus producing ability is implicated in the bacterial adherence at the very beginning of the nasopharyngeal colonisation and at the same time may also be the triggering factor for the inflammation of the mucous membrane causing damage to it and consequently facilitating pneumococcal penetration into the tissue and the development of infection. The aim of the study was to determine the prevalence of the pilus in a set of invasive strains covered by 7-valent pneumococcal conjugate vaccine (PCV7).
Methods: A total of 135 pneumococcal invasive isolates of 7 serotypes recovered from blood (n = 82) and cerebrospinal fluid (n = 53), were studied. The isolates were of the following serotypes: 4 (37), 6B (18), 9V (27), 14 (4), 18C (11), 19F (6), and 23F (32). Serotyping was performed by the Quellung reaction and all isolates were analyzed by MLST. The presence of pilus was performed by PCR of the sortase B, C, and D genes which are a structural part of the rlrA pathogenicity islet. Confirmation of pneumococcal pilus absence was done by amplification of the entire pilus islet with primers PFL-up and PFL-dn and comparison of the obtained PCR product with R6 DNA as non-piliated control.
Results: The 7 serotypes were classified into 34 sequence types (ST), range 2 to 12 ST's per serotype. Except ST808 in serotype 4, all remaining ST's possesed at least 2 isolates. More than half of isolates were positive for the presence of sortases genes (n = 71, 52%). The pilus was found in serotypes 4, 6B, 9V, 14, and 19F. The presence of the pilus was highly congruent with serotypes as well as clonal types. Only 3 out of 33 clonal groups were composed of isolates that differed in the presence of the rlrA islet.
Conclusions: With the exception of serotypes 18C and 23F, all paediatric serotypes covered by PCV7 were capable of formation of the pneumococcal pilus. The high prevalence of the pilus in invasive isolates indicates that the pilus play a role in human infections.
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P1344 Emergence of a clone of *Streptococcus pneumoniae* serotype 19A in children in Murcia, Spain

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Background: Serotype 19A is know to have risen as important cause of invasive pneumococcal disease (IPD) after the introduction of the heptavalent pneumococcal conjugate vaccine (PCV7). During routine surveillance of serotype distribution of paediatric invasive *S. pneumoniae* isolates in Murcia (Spain), we detected an increased of 19A serotype. The objective of this study was to describe *S. pneumoniae* serotype distribution in our geographic area in children <11 years with emphasis on serotype 19A.
Methods: From March 2006 to April 2008 we studied 40 isolates of *S. pneumoniae* obtained from a normally sterile site (IPD) from children attending in Hospital Virgen Arrixaca, Murcia. Serotyping was made by Quellung reaction. Susceptibility to penicillin (P), cefotaxime (CTX), erythromycin (ER), levofloxacin (LV), chloranphenicol (C), tetracycline (TE) and vancomycin (VA) was determined by E-test and interpreted according to CLSI guidelines. Molecular typing of 19A isolates was performed by Pulsed Field Gel Electrophoresis (PFGE) using SmaI endonuclease.
Results: The most common serotypes were the non-PCV7 serotype 19A (8, 20%) and the PCV7 cover serotype14 (8, 20%). The distribution of

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 whit bacteraemia (16.7%) and bacteraemia (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 $\geqslant$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 non-β-lactam agents were as follows: E 25/37.5; TE 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 commoly used antimicrobial was greater than the proportion for other serotypes.

P1345 Modified sequential multiplex PCR for determining capsular serotypes of invasive *Streptococcus pneumoniae* clinical isolates

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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 Sevilla, Andalucía (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 targeting all known pneumococcal cps operons. The main modification was to include primer pairs for the 1 and 5 serotypes in the first three reactions.

Results: In the paediatric isolates group, the concordance of the PCR results with conventional serotyping was 91%. Three of the 5 isolates for which serotypes could not be deduced were serotypes not included in the multiplex reaction scheme. The multiplex PCR typed 97.4% (151/155) of adult's isolates, and 93% (95/102) of paediatric isolates. In adult isolates, the modified multiplex PCR scheme, allowed us to type 73% of them, and with two additional PCRs 92%, while using the original scheme we would type 63% and 74.8% of the isolates respectively. In the paediatric isolates group, results obtained were the 73.5% versus 60%, with the first three reactions and 90% versus 72.5% with the two additional PCRs.

Conclusion: The modified sequential multiplex PCR is better adapted to the serotype distribution of invasive *Streptococcus pneumoniae* from our geographic area and this scheme, improve the efficiency of original CDC seven multiplex PCR.

P1346 Insight into the genetic diversity of multidrug-resistant *Mycobacterium tuberculosis* in southern Brazil

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We present a picture of the biodiversity of *Mycobacterium tuberculosis* in the Southern Brazilian state of Rio Grande do Sul. This state harbours more than 10 million inhabitants with a tuberculosis prevalence rate of 41/100,000 in 2007. The objective of this study was to genotype the multidrug-resistant *M. tuberculosis* in order to improve our understanding of tuberculosis (TB). Spoligotyping was chosen due to its fast generation of genotyping results, high reproducibility and low operating costs. SpolDB4 database was used to assign the isolates to families, subfamilies and variants. The results of a study can thus be analyzed in a more global context. All the strains of *M. tuberculosis* genotyped were isolated from patients with pulmonary TB who had a positive acid fast bacilli smear. Heat-killed samples were DNA extracted as described by van Soolingen et al, and samples were spoligotyped according to Kamerbeek et al. The samples from this population-based molecular epidemiology study were obtained from 148 consecutive TB cases in Rio Grande do Sul. Patients were 61.5% male and 38.5% female. Of these, 71.8% were tested for HIVusing the ELISA method and 28.2% were seropositive. The percentages of HIV cases between male and female were 61.2% and 38.7% respectively. Rifampicin (R) and isoniazid (H) resistance were detected in 114 (77%) strains. In 35 (23%) strains, we detected other associated resistance, such as RH + Streptamicin (SM) in 20 cases (13.6%) and RH + ethambutol (EMB) in 8 cases (5.3%). There were 6 related MDR-TB cases (4.1%) that were resistant to R + H + SM + EMB. The molecular analysis gave 35 different spoligopatterns that presented an overall diversity of 48%: 17 spoligopatterns occurred only once and the remaining 18 patterns were associated with 131 of the isolates (88.5%). Seven of the 35 patterns had not been described previously. The prevalence of the MDR-TB lineages in this study was: Latin-American & Mediterranean (LAM) family: 65%; T family: 17.9%; U family: 12.1%; Haarlem family: 3.6%; X family: 1.4%. Spoligotyping is very useful in gaining an overall understanding of the TB epidemic. This study demonstrated that the MDR-TB situation in Rio Grande do Sul, Brazil was caused by only a few *M. tuberculosis* families of which the most prevalent was the RH-resistant LAM family commonly found in Mediterranean region.

P1347 Molecular typing of *Legionella pneumophila* from a hospital's water system

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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. 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/lt. 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 rtxA. During the study period, no 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

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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 ⩾3 of: expanded-spectrum β-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: *Citrobacter 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 up to 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 repFIIAs), 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 repF replicons. The following incompatibility groups were identified in *C. freundii*: IncF (positive for repFIC) and IncP (C1), IncHI2 (C2) and IncHI1, repF and IncFIIAs (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*.
Conclusions: Most multiresistant enterobacteria other than *E. coli* (except *M. morganii*) isolated in our area contains plasmids of different incompatibility groups. Similar plasmids have been observed in isolates of the same species but of different clonal groups.

P1349 Replicon typing of plasmids coding for β-lactamases of the CTX-M-9 group or CMY-2 in multiresistant *E. coli* clinical isolates from Cantabria, Spain

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Objectives: In an ongoing study on multiresistant *E. coli* producing (n = 100) or not (n = 100) extended-spectrum β-lactamases (ESBL) isolated in Santander, Spain, 21 clonally-unrelated (as determined by Rep-PCR) isolates producing β-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/sulfametoxazole (100 mg/l, 8 mg/l and 1000 mg/l; blaCMY-2), respectively. Plasmid 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 β-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 Inc I1 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 β-lactamase genes with specific large conjugative plasmid: those coding for blactx-m-9-group are of IncK and Inc I1 incompatibility groups, while blaCMY-2 is related to plasmids of IncA/C2 and Inc I1 groups.

P1350 Characterisation of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates causing an outbreak in an intensive care unit

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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, amikacine and ciprofloxacin. ESBL PCR was performed on 107 of the 118 strains. In 79 isolates the SHV-amplicon was digested with NheI and was therefore regarded as ESBL-positive. 107 out of 118 showed the presence of an integron with an amplicon 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

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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 polymorfisms (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* (MRD-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.

P1352 Rapid and effective analysis of nosocomial outbreaks using the DiversiLab semi-automated repetitive sequence-based PCR test system

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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-β-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 (8–24 h), which is an essential advantage in rapid analysis of hospital associated infections.

P1353 Optical typing in bacterial epidemiology using Raman spectroscopy

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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 20 hr on Trypticase Soy agar plates. Biomass was suspended in 10 microliter of sterile distilled water, transferred onto a quartz slide and allowed to dry. Spectroscopic fingerprints were obtained using a dedicated Raman spectrometer, requiring 10 to 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.

P1354 Molecular and epidemiological plasmid profile analysis of *Enterococcus faecalis* isolates

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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 fimbriae fim2 and fim3 genes in the Finnish *Bordetella pertussis* population

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Objectives: *Bordetella pertussis*, the causative agent of pertussis produces three distinct serotypes: Fim2, Fim3 or Fim2,3. These fimbriae are coded by the fim2 and fim3 genes, level of fimbrial expression determines the serotypes of *B. pertussis*. The fimbriae 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 fimbrial gene alleles of Finnish *B. pertussis* isolates collected since 1990 s.
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

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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, cmxA/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 −80ºC 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

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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 marker(s) in medically important fungi.
Methods: Clinical *Candida* isolate, susceptible to azole antimycotics was subcultured on agar plates with increased concentrations of tested antimycotics (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, adaptor 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 antimycotic 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 antimycotic resistance in medically important fungi.

P1358 Molecular epidemiology of invasive meningococcal disease in the Czech Republic

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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 characterisation 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 FetA sequencing (http://neisseria.org/nm/typing/) and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/).
Results: IMD in the Czech Republic in 2007was caused mainly by serogroup B (67.4%), followed by serogroups C (20.9%) and Y (9.3%). The following clonal complexes were most frequently associated with IMD: cc11, cc18, cc41/44 and cc32 (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 (18.4%) and a high proportion of strains not assigned to clonal complex (39.9%). In 2007, invasive meningococcal disease occurred in 49 out of 86 districts. A single case was reported in 30 districts, two or more cases occurred in 19 districts. No secondary case of IMD was detected by the analysis of epidemiological data and isolate genotypes. Genotypes were identified for 8 clusters of pair case/contact isolates. The case and contact isolates had identical genotypes in 3 clusters, the former differed in genotype from the latter in other 3 clusters and mixed genotypes were observed in 2 clusters. All pair case/close contact isolates had identical genotypes in contrast to most pair case/non-close contact isolates that differed in genotype.
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.

P1359 Genotypes of *Coxiella burnetii*. Comparison between AFLP and VNTR typing strategies

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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 labeled restriction fragments were separated on 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. burnettii* genotypes. Only two VNTR markers demonstrated polymorphisms in the Slovak isolates.
Results: The VNTR typing demonstrated that the *C. burnettii* isolates from Slovakia form quite a homogeneous genotypic cluster. Both typing methods, VNTR and AFLP, have demonstrated that the *C. burnettii* genome is conservative and evolutionary stable. The generation of polymorphisms is not dynamic. It appears that *C. burnettii* 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 patter 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. burnettii* isolates a specific PCR could be developed for unique marker(s).

P1360 Direct genotyping of *Coxiella burnettii* in human and animal clinical samples

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Objectives: Studies towards the molecular epidemiology of *Coxiella burnettii* are hampered by the availability of sufficient material for analysis. So far, molecular fingerprinting of *C. burnettii* 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. burnettii* 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 analyzed 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. burnettii* 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 $\leqslant$ 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. burnettii* and to identify the possible origin of human Q-fever infections.

P1361 Molecular typing of *Listeria monocytogenes* virulent serotypes

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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-fatally 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

belonging to the same serotype are greatly required for epidemiological surveillance. The repetitive extragenic palindromic element-based PCR (REP-PCR) is rapid, as discriminatory as PFGE and has a typing potential similar to RADP method. In this study we have evaluated the utility of an automated rep-PCR method for genomic fingerprinting of *L. monocytogenes* strains belonging to serotypes 1/2a, 1/2b and 4b.
Material and Methods: Ninety-five *Listeria monocytogenes* and five non-*monocytogenes Listeria* strains isolated from different sources were studied. Identification was confirmed by multiplex PCR using specific primers to amplify a 938 pb fragment from 16S rDNA of *Listeria* spp. and a 750 pb fragment from hlyA gene of *L. monocytogenes*. Biotype was determined by API-*Listeria* system. Serotyping was performed by heat-inactivated bacteria agglutination with the commercial system *Listeria* antisera set (Denken Seiken.Co, Ltd., Tokio).
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.
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P1362 Molecular typing of human isolates of *Listeria monocytogenes*

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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, ApaI, AscI, and SmaI.
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 prfA 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 Hys 181 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.

P1363 *Chlamydia trachomatis* genotypes in a group of male patients coming from a sexually transmitted diseases clinic in a city in northern Spain

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Objectives: *Chlamydia trachomatis* is one the most prevalent sexually transmitted bacterial pathogen. Serovars D to K are commonly associated with urogenital infections in males such as uretrithis and epididymitis. Lymphogranuloman 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 pb-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 Teminator 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 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 (18.7%), 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%).
Conclusions: 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.

P1364 Genotyping of *Chlamydia trachomatis* in Bilbao, Spain

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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 STI Laboratory for assessment between January 2007 and June 2007 were screened as positive for *C. trachomatis* by polymerase chain reaction (PCR) using and 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 uL 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%), J (7.31%), H (10.97%), Ia (4.87%), L2 (1.21%), and L2b (3.66%). Patients with *C. 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 in other studies, but further research in this point is necessary.

P1365 Detection and genotyping of *Chlamydia trachomatis* in clinical urogenital samples from north-eastern Croatia

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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 chlamydia 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 genitourinary infection considering age and sex and therapy efficiency, as well as possible presence of chlamydial coinfection and reinfection.

P1366 Molecular analysis of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains, isolated from intensive care unit patients of with hospital-acquired pneumonia

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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 Bio1D++ (Vilber Lourmat, France). Genetic similarity between strains was viewed by using dendrograms. The dendrograms with UPGMA clusterisation 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 (appropriately 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.

P1367 Molecular typing of a new variant of *Encephalitozoon cuniculi* responsible for disseminated microsporidiosis in a renal transplant recipient

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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, urines 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 urines, 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.

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

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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 life style 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 calve 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

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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%).
Preliminary 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.

P1370 Methicillin-resistant *Staphylococcus aureus* Clonal Complex 398 does not spread from farms into the community

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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 at 450 persons without livestock contact to exclude a prevalence of at least 2%, assuming a background prevalence of 0.5% (α=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 (8/39: 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).

P1371 Detection of *Staphylococcus aureus* multi-locus sequence type 398 (ST398) in blood cultures

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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 bacteraemia 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 bacteraemic patients in an area in The Netherlands with a high density of pigs.

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 were checked for the unique ST398 banding pattern.
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.

P1372 Heterogeneity among porcine MRSA ST398 isolates

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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 isolate were screened using diagnostic microarrays.
Results: Fifty-three strains harboured SCCmec type V elements while the remaining one carried mecA and ugpQ, but no recombinase genes. β-lactamase genes (blaZ, blaI, blaR) were found in fifty-three strains. Macrolide/lincosamide resistance genes ermA, ermB and ermC were found in five, seven, and thirteen strains, respectively. The streptogramin resistance gene vga(A) was detected in seven strains, the aminoglycoside resistance genes aacA-aphD in eight and aadD in nine strains. Tetracycline resistance genes were commonly found with tetK in fifty-one and tetM in fifty-three strains. Usually, both tet genes were simultaneously present in the ST398-MRSA-V strains. Chloramphenicol resistance was rare (fexA in two strains). Some strains carried enterotoxin genes which might be of relevance in the pathogenesis of diseases in humans. One strain was positive for the enterotoxin B (seb) gene, another three strains for the enterotoxin K and Q (sek, seq) genes. None of the isolates harboured the Panton-Valentine leukocidin genes lukF-PV and lukS-PV or the genes sak, chp, and scn indicative for haemolysin beta converting phages. The carriage of haemolysin alpha and delta genes, the protease genes sspA, sspB, and sspP, the ssl and set genes, as well as MSCRAMM genes was uniform among all strains.
Conclusion: The microarray results revealed a high degree of diversity regarding resistance genes and virulence determinants. These data might point towards a tendency of the ST398-MRSA-V strains to readily acquire resistance genes commonly associated with mobile genetic elements such as plasmids or Transposons. Moreover, the presence of enterotoxin genes seb, sek and seq could indicate an evolutionary trend towards an increased pathogenicity for humans.

P1373 Longitudinal evaluation of methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* in pigs

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Objectives: The potential public health concerns of MRSA and *Clostridium difficile* in food animals are receiving increasing attention, yet little is known about the dynamics of colonisation in pigs. The objectives of this study were to characterise the prevalence of MRSA and *C. difficile* colonisation in piglets over time on commercial swine farms.
Methods: One farm was evaluated for each organism. For MRSA, nasal swabs were collected from sows 2 weeks prior to farrowing, then from piglets from 3 MRSA positive and 7 MRSA negative sows at 1, 3, 7, 14, 21, 28, 42, 56 and 70 days of life. Enrichment culture for MRSA was performed. For *C. difficile*, sows were sampled 30, 22 and 15 days prior to farrowing and rectal swabs were collected from piglets from 5 positive and 5 negative sows on days 2, 7, 30, 44 and 62 for enrichment culture and typing.
Results: MRSA colonisation rates were low initially, but increased over time, with 79% of piglets positive on at least one occasion. The prevalence of MRSA colonisation on days 1, 3, 7, 14 and 21 was 1%, 6.2%, 8.5%, 4.4%, 20%, respectively, with 35% of piglets positive prior to weaning. 34%, 64%, 50% and 41% of pigs were colonised on days 28, 42, 56 and 70, respectively. Of piglets surviving to weaning 84% of piglets from negative sows and 100% of piglets from positive sows were positive on at least 1 sample. A piglet from an MRSA positive sow was 1.4 times more likely to be colonised than a piglet from a negative sow ($P = 0.037$). All MRSA isolates were PFGE non-typable and spa t034. In contrast, *C. difficile* colonisation rates were high initially and decreased over time. *C. difficile* was isolated on one or more occasions from 97% (117/121) of piglets. The prevalence of colonisation on days 2, 7, 30, 44 and 62 was 74%, 56%, 40%, 23% and 3.7%, respectively. 133/143 (93%) tested isolates were ribotype 078/toxinotype V.
Discussion: Colonisation rates were high for both pathogens, but the contrasts between them were interesting. The reasons for the changes in prevalence over time are unclear and require investigation to determine whether factors can be modified to reduce the risk of transmission. The use of single farms limits making broad conclusion, but this study provides insight into age-related effects on colonisation that should be considered in future studies. While colonisation status of the sow influences the piglets, there is not an absolute relationship and other sources of infection need to be considered.

P1374 Evaluation of *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus* contamination of retail chicken meat

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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 CDMN 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; 8/50 (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.

P1375 Virulence and resistance determinants in methicillin-resistant *Staphylococcus aureus* ST398 isolates

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Objective: Detection of virulence and resistance genes in German MRSA isolates of the MLST clone ST398.
Methods: A series of 106 *S. aureus* isolates were selected. They included 100 MRSA (2004–2007) of the ST398 clone, and six out-group/control strains. The isolates originated from healthy and sick pigs, dust from pig farms, milk, carcasses and meat from food-producing animals. They 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 SmaI-PFGE, and screened for thirty seven genes involved in virulence and eight involved in tetracycline or erythromycin resistance by PCR.
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, hld ± hlg) and were of the agr quorum sensing system type I. Out-group and control isolates displayed different virulence profiles and were from other agr groups. All the isolates of the clone were tetracycline resistant and carried the gene tet(M) together with one or two plasmid associated genes [tet(K) and tet(L) (18%), tet(K) (51%), tet(L) (22%)]. For erythromycin and clindamycin resistance, eight patterns [ermA, ermB, ermC (8.2%), ermA, ermB (19.2%), ermA, ermC (8.2%), ermB, ermC (1.4%), ermA (11%), ermB (12.3%), ermC (35.6%), non-typeable (4.1%)] were found among resistant isolates. A relationship between the presence of certain resistance genes and the SCCmec type was detected. Most of the SCCmec III isolates carried ermA, which is associated with the Tn554 transposon present in this cassette. The plasmid gene tet(K) was found only in isolates with SCCmec V.
Conclusions: The ST398 isolates from swine, milk, carcasses and meat lack several clinically important *S. aureus* associated virulence factors, including PVL. Generally, only some haemolysin encoding genes and the agr type I were present. Other virulence mechanisms related to the adhesion or production of biofilms may play an important role in the wide spread of ST398 isolates. All MRSA ST398 were tetracycline resistant and carried the tet(M) gene with additional resistance genes mediated by plasmids (tet(K) and/or tet(L)). Most isolates (69%) were erythromycin-clindamycin resistant and carried different combinations of erm genes.

P1376 Animal-associated ST398 MRSA strains lack human-associated virulence and adhesion genes

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Background: In Belgium, national epidemiological surveillance of methicillin resistant *S. aureus* (MRSA) strains isolated from hospitalised patients showed that nearly 90% belong to nine predominant PFGE types, belonging to well known international pandemic MRSA lineages, MLST Clonal Complex (CC) 45, CC8, CC5 and CC22. More recently, ST398 MRSA strains have been described as agent of infection or colonisation in horses, pigs, poultry and humans.
Objective: The aim of the present study was to detect, by the DNA-microarray technique, virulence and resistance genes possessed by ST398 "animal-associated" MRSA strains compared to strains belonging to well known hospital acquired (HA-) MRSA clones.
Methods: Representative strains of the most frequent Belgian HA-MRSA epidemic clones (n = 9) and ST398 strains (n = 16) were selected from the National Reference Laboratory for Staphylococci collection. ST398 strains were selected from a wide range of host species (horse, pig, poultry and human). These strains were characterised by a microarray designed among 8 *S. aureus* sequenced genomes and composed of 390 oligonucleotide probes targeting resistance (37%), virulence (31%) and adhesion factors (32%).
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 CCs. 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 (embbp, 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 human – and raise the issue of its colonising and infecting capacity for humans.

P1377 Methicillin-resistant *Staphylococcus aureus* CC398 in humans and animals, Finland

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Objective: Animals may serve as a reservoir for methicillin-resistant *Staphylococcus aureus* (MRSA). In Denmark and the Netherlands, the MRSA lineage CC398 is common among pigs (10–40%). In Finland, the occurrence of MRSA among pigs is under investigation, so far one isolate has been found. In 2007, an epidemic of CC398 occurred in a veterinary hospital involving 11 horses and one employee. During 2008, CC398 was recognized in six other humans. To recognize the possible connections among CC398 clusters, the background information from persons with CC398 is enquired by a questionnaire, and all the CC398 isolates recovered from humans and animals are characterised in detail by molecular methods.
Methods: The plausible CC398 isolates were initially recognized by their non-typability in SmaI-pulsed field gel electrophoresis (PFGE). Further analysis was performed by spa typing, multilocus sequence typing (MLST), Staphylococcal chromosome mec (SCCmec)-typing, detection of Panton-Valentine leukocidin genes (PVL), ApaI-PFGE, and antimicrobial resistance testing. The background data enquired included presence of preceding animal contacts and known risk factors for MRSA, and whether the specimen was obtained because of symptomatic infection or screening.
Results: The seven human findings, with no apparent connections between each other, were from three health care districts. All seven isolates were resistant to tetracycline, but resistance to aminoglycosides, macrolides and clindamycin was variable. All human and animal isolates analyzed thus far, fell into three spa types: the horse isolates and two human isolates from separate health care districts were spa type t011, two human isolates from the same health care district were spa type t034, and the pig isolate was spa type 108. The t034 isolates

harboured PVL-genes. By ApaI-PFGE, three clusters, separated by 5–7 band differences, were found: 1) t034 human isolate, 2) t011 horse isolate and the epidemiologically related human isolate, and 3) the other t011 human isolate and the t108 pig isolate. Other typing results and the background information will be presented.
Conclusion: Subtyping of human and animal MRSA suggests non-common source for recent emergence of CC398 MRSA in humans in Finland. However, clustering of human and animal isolates was detected. Further epidemiological background information is required to reveal the transmission chains.

P1378 First infections by methicillin-resistant *Staphylococcus aureus* sequence type 398 in Spain

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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 paticularly 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 where 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 accesory 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, SCCmec-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.

P1379 Comparison of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* infections in dogs and cats

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Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) is emerging as an important pathogen in companion animals, but there has been minimal objective comparison of MRSA infections with those caused by methicillin-susceptible *Staphylococcus aureus* (MSSA) infections. The objectives of this study were to compare the infection types, clinical outcomes, and determine the risk factors associated with MRSA, compared to MSSA infections, in dogs and cats.
Methods: A retrospective case-control study was conducted at 3 veterinary referral hospitals. An MRSA infection was identified and was matched by species, veterinary referral hospital, and date of admission to 2 MSSA controls: the MSSA infection immediately preceding and following the MRSA case. A questionnaire was used to collect information from the medical record of all cases and controls. Data were collected concerning signalment, medical and surgical history, infection, and clinical outcome. Outcomes were defined as animals having an MRSA or MSSA infection. Analyses were performed using exact logistic regression. Due to the sample size, a multivariable model could not be constructed due to concerns of model stability and issues associated with over-fitting the model. Consequently, only univariable models were constructed.
Results: A total of 46 MRSA cases and 92 MSSA controls were enrolled consisting of 120 (86.9%) dogs and 18 (13.1%) cats. The largest proportion of MRSA and MSSA infections were located on the skin 58.7% (27/46) and 64.4% (58/90), respectively. The majority of animals with MRSA (93.3%, 42/45) and MSSA (91.1%, 81/89) infections were discharged from the hospital. Antimicrobial administration (OR = 3.55; 95% CI: 1.22–11.94; P = 0.014) and fluoroquinolone administration (OR = 3.77; 95% CI: 1.03–17.03; P = 0.037) were significantly associated with the development of an MRSA infection.
Conclusion: This study is the first to objectively compare MRSA and MSSA infections in dogs and cats and identify risk factors for the development of an MRSA infection. The identification of antimicrobials, specifically fluoroquinolones, as risk factors for MRSA infection supports the need for prudent use of antimicrobials in pets. The high survival rate indicates that MRSA is largely a treatable infection, likely because of the high frequency of non-invasive infections. While no difference in outcome for MRSA and MSSA infections was observed, further study of invasive infections is warranted.

P1380 Microbiological characterisation of *Staphylococcus pseudintermedius* isolates from dogs

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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 the 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.

Date	Number of MRSA positive animals/Total number of animals tested (% MRSA positive animals)	
	Dolphins	Walruses
February 2007	2/6 (33.3%)	2/5 (40%)
April 2007	2/5 (40%)	0/6 (0%)
May 2007	2/3 (66.7%)	0/6 (0%)
October 2007	1/5 (20%)	0/5 (0%)
May 2008	1/5 (20%)	–
July 2008	0/5 (0%)	–
October 2008	0/5 (0%)	–

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.

P1382 Methicillin-resistant *Staphylococcus aureus* colonisation of veterinary personnel at a surgical conference

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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 surgeon 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 6/33 (18%) 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. ST398 strains were not isolated.
Discussion: The colonisation rate in this study was striking and provides further support suggesting MRSA exposure is an occupational risk for veterinary personnel. Unlike earlier studies, there were no differences between small animal and large animal personnel. The association of MRSA and small ruminant contact was unexpected and requires further investigation. The association with a person in charge of infection control presumably reflects the fact that facilities with infection control personnel at inherently at higher risk as opposed to a negative impact of infection control programs.

P1383 Emergence and differentiation of pig-associated methicillin-resistant *Staphylococcus aureus* CC398 in Austria

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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 (BioMerieux).
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 and trimethoprim/sulfamethoxazole.
Determination of the genetic relatedness using rep-PCR on the Diversilab System surprisingly resulted in one single cluster for all MRSA CC398 isolates from 2002–2007.

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 carriers

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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 SmaI restriction enzyme, MLST analysis, spa-typing, agr-typing and SCCmec typing. Additionally, the detection of genes encoding virulence factors, such as enterotoxins (sea-see, seg-sei), exfoliative toxins (eta, etb), tsst, 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

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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 biotyping.
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 enterotoxigenic 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

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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, cefoxin, 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 ciprofloxacine against intracellular *Brucella melitensis*

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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 ciprofloxacine 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 37ºC 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 37ºC 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 and the bacterial colonies enumerated. The results of each series (n = 8) of experiments are presented as the means values of the bactericidal percent compared with a control without antimicrobial.
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 the both antibiotics accumulates in the granulocytes, increasing their bactericidal activity.

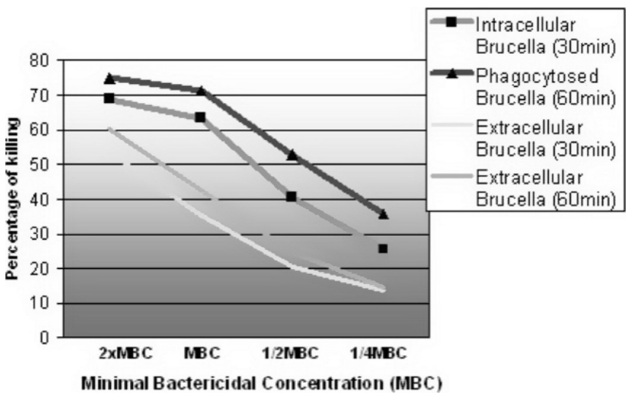

Figure 1. Effect of ciprofloxacin in the killing of phagocytosed *Brucella*.

P1388 In vitro activity of tigecycline, doxycycline, streptomycin, rifampicin, and ciprofloxacin against 70 strains of *Brucella melitensis*

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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 37ºC 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/I.
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

Antibiotic	Range (mg/l)	MIC_{50} (mg/l)	MIC_{90} (mg/l)
Tigecycline	0.064–1	0.38	0.5
Doxycycline	0.047–1.5	0.19	0.25
Streptomycin	0.25–1	0.5	1
Rifampicin	0.5–32	1	2
Ciprofloxacin	0.125–32	0.38	0.5

P1389 Anti-*Helicobacter pylori* and anti-internalisation activities of Thai folk remedies used to treat gastric ailments

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Helicobacter pylori, an aetiological agent of active chronic gastritis and peptic ulcer disease, is now considered to be as invasive enteropathogen. Invasion of gastric epithelium cells contribute to persistence 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 ug/ml and 64 ug/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.

P1390 In vitro activities of 22 antimicrobial agents against macrolide-resistant *Campylobacter jejuni* and *Campylobacter coli* isolates

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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 from 19 erythromycin-resistant and 25 erythromycin-susceptible strains by pyrosequencing.
Results: Of all 232 *Campylobacter* isolates, 19 were resistant to erythromycin (MIC $\geqslant$ 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 19 erythromycin-resistant *Campylobacter* strains had a 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.

P1391 Antimicrobial susceptibility of genital *Mycoplasma hominis* and *Ureaplasma urealyticum*

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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, clarythromycin, 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, clarythromycin (*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)

Antimicrobial agent	S, n (%)	I, n (%)	R, n (%)
Doxycycline	79 (98.8)	0	1 (1.2)
Erythromycin	68 (85)	6 (7.5)	6 (7.5)
Josamycin	80 (100)	0	0
Ofloxacin	34 (42.5)	28 (35)	18 (22.5)
Tetracycline	77 (96.3)	0	3 (3.7)
Ciprofloxacin	19 (23.8)	16 (20)	45 (56.2)
Azithromycin	68 (85)	6 (7.5)	6 (7.5)
Clarithromycin	70 (87.5)	3(3.8)	7 (8.7)
Pristinamycin	79 (98.8)	1 (1.2)	0

S: susceptible, I: intermediate, R: resisant.

Table 2: Antimicrobial susceptibility testing of *M. hominis* (n: 5)

Antimicrobial agent	S, n (%)	I, n (%)	R, n (%)
Doxycycline	5 (100)	0	0
Erythromycin	1 (20)	0	4 (80)
Josamycin	5 (100)	0	0
Ofloxacin	4 (80)	0	1 (20)
Tetracycline	4 (80)	0	1 (20)
Ciprofloxacin	3 (60)	1 (20)	1 (20)
Azithromycin	1 (20)	1 (20)	3 (60)
Clarithromycin	1 (20)	0	4 (80)
Pristinamycin	5 (100)	0	0

S: susceptible, l: intermediate, R: resistant.

P1392 Antibiotic susceptibilities of genital mycoplasmas in male patients with urethritis

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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 doxycycline was 5.8%. For *Mycoplasma hominis*, 11 (73.3%) and 7 (46.7%) isolates were resistant to ciprofloxacin and ofloxacin respectively. Resistance against azithromycin, clarithromycin, erythromycin, josamycin and pristinamycin were 66.7%, 100%, 100%, 0% and 0% respectively. No resistance was observed against doxycycline.

Conclusion: Fluoroquinolones resistance was very high among *Mycoplasma hominis* and Ureaplasma ureayticum isolates. Macrolides resistance was low in *Ureaplasma urealyticum*. For *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*

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Background: Lower respiratory tract infections apart from being caused principally by *Streptococcus pneumoniae* and *Haemophilus influenzae*, are increasingly being caused by methicillin-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, 81 penicillin intermediate, and 94 penicillin resistant strains: 120 were erythromycin resistant (MICs >1 mg/L)[(65 erm(B), 32 mef(A), 1 erm(B) and mef(A), 19 L4 and 3 23S rRNA)]. The 150 *H. influenzae* (146 untypeable, 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 contaning 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 s 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

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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 transporter during operation and subjected to bacterial culture within 24–48 hrs. Specimens were plated onto several aerobic and anaerobic media (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. Carbapenems 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: Carbapenems 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

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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 aseptic microbiological methods, 220 delayed-healing diabetic-foot ulcer samples were analyzed for bacteria isolation, identification and susceptibility test. Chi-square (á=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 Levofloxacin and Sparfloxacin respectively. *P. aeruginosa* was also: 68.7% susceptible to rifampicin; 53.0% to erythromycin, 52.2% to vancomycin; 38.3% to ceftriazone; 36.5% to cefuroxin; and 32.2% to oxacillin. *S. aureus* was: 51.7% susceptible to rifampicin, 37.1% to cefuroxin; 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 1040:2006

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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

Methods: Several products from market have been investigated: Chlorchinaldin, Cholinex, Cholisept, Halset, Menthosept, Neo-angin, Orofar, Propolki, Sebidin, Sebidim Plus, Septolete D, Septolete Plus, Strepsils, Strepsils Intensive, and Tantum Verde.
Basic bactericidal activity of preparations was analyzed according to European Standard EN 1040:2006, with additional test organisms recommended by prEN 13727. Appropriate standard strains of *S. aureus* ATCC 6538, *E. hirae* ATCC 1541, *E. coli* NCTC 10538 and *P. aeruginosa* ATCC 15442 were used. Two contact times (15 min and 1 h) and bacterial suspensions density of $1.5–5\times10^8$ 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 (5-log) of cells count of four tested bacterial strains was achieved, not only after 1 h but even after 15 min of contact time. In case of Cholinex, Cholisept, Propolki, Sebidin, Septolete D, Septolete Plus and Tantum Verde, 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 oral antiseptics 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 *Corynebacterium macginleyi*: susceptibility to 7 usual ophthalmic antibiotics

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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, vancomicin, 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 days.
Identification was performed using the API Coryne system (bioMérieux). Susceptibility was determined by microdilution broth method according to CLSI breakpoints and using its recomendations as well: Cation-adjusted Mueller-Hinton broth with lysed horse blood (2.5 to 5% v/v), 35ºC, aerobic atmosphere and 24 to 48 hours of incubation.
Results: After 48 hours of incubation all the strains were susceptible to penicillin ($\leqslant$1 mg/l), gentamicin ($\leqslant$4 mg/l), vancomicin ($\leqslant$4 mg/l), rifampicin ($\leqslant$1 mg/l) and linezolid ($\leqslant$2 mg/l). Only one strain was resistant to ciprofloxacin (16 mg/l $\geqslant$ 4 mg/l) and another to tetracycline (64 mg/l $\geqslant$ 16 mg/l).
MIC50 and MIC90 obtained were as follows: $\leqslant$0.06 mg/l and $\leqslant$0.06 mg/l, $\leqslant$ 0.06 mg/l and 0.25 mg/l, $\leqslant$ 0.06 mg/l and 0.25 mg/l, 0.5 mg/l and 1 mg/l, 0.5 mg/l and 1 mg/l, $\leqslant$0.06 mg/l and$\leqslant$ 0.06 mg/l, 0.25 mg/l and 1 mg/l to penicillin, gentamicin, ciprofloxacin, tetracycline, vancomicin, rifampicin and linezolid, respectively.
Conclusions: *Corynebacterium* macginleyi showed high susceptibility to all the ophthalmic antibiotics tested.
Only two strains were found to be resistant to one of them: ciprofloxacin or tetracyclin.
From these data we conclude that antimicrobial treatment of *C. macginleyi* obtained from ophthalmic samples is not an important problem, at least for the time being.

P1398 In vitro activity of co-trimoxazole and comparators against Gram-positive and negative blood stream pathogens

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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), ciprofloxaxin (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 number of strains tested and percent susceptible for Gram negatives and Gram positives are shown (Table). The proportion of Enterobacteriacae 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.

Species	n	% Susceptible							
		Co-t	AMP	CoA	CXM	CTRI	CIP	GEN	ERT
E. coli	191	60	35	64	79	88	73	80	99
Ent. cloacae	30	83	0	0	53	50	83	90	90
K. pneumoniae	47	60	0	45	53	62	55	81	98
S. marcescens	16	75	0	6	6	50	69	100	94
Proteus	28	71	82	79	100	100	100	100	100
Other Enterobacteriaceae	23	87	0	48	65	83	78	91	96
All	335	66	27	56	69	80	74	84	98

Species	n	% Susceptible							
		Co-t	AMP/P	VAN	DAP	LIN	CIP	FUS	ERY
MRSA	98	95	–	100	100	100	9	89	31
MSSA	68	98	–	100	100	100	24	92	90
CNS	24	71	–	100	100	100	58	58	50
E. faecalis	52	7	81	98	100	100	–	–	–
E. faecium	28	4	10	85	100	100	–	–	–
BHS (ABCG)	32	100	100	–	–	–	–	–	–

P1399 In vitro susceptibilities of *Mycobacterium marinum* isolates in Taiwan

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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

Pathology Laboratory, Chang Gung Memorial Hospital, Lin-Kuo Medical Center from Oct 1, 1999 to Mar 31, 2008.
Susceptibility tests: We use 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 $\leqslant$ 1 mg per L; EMB $\leqslant$ 5 mg per L; CLR $\leqslant$ 16 mg per L; DOX $\leqslant$ 4 mg per L; SMX $\leqslant$ 32 mg per L; AMK $\leqslant$ 32 mg per L; LZD $\leqslant$ 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.

Antibiotic	MIC (mg/L)			% Susceptible
	50%	90%	Range	
RIF	1	4	0.5–4	84%
EMB	0.5	4	0.25–4	100%
CLR	4	8	2–8	100%
DOX	16	32	8–32	0%
SMX	4	64	0.5–64	92%
AMK	4	16	1–16	100%
LZD	2	4	0.5–4	100%

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.

P1400 Comparative in vitro activities of moxifloxacin and 6 other antimicrobial agents against aerobic bacterial isolates causing intraabdominal infection: results from the PRISMA study

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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/sulbactam (AMP/SUL); piperacillin/tazobactam (PIP/TAZ); ceftriaxone (CTX); ertapenem (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.
Conclusions: 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*. Ertapenem and meropenem showed the highest in vitro activity against most bacterial species obtained from IAI.

	MIC50/90						
	AMP/SUL	PIP/TAZ	CTX	ERT	MER	LEV	MOX
Citrobacter spp.	4/⩾256	2/64	0.25/64	⩽0.03/0.12	⩽0.03/0.12	0.06/1	0.12/2
Enterobacter spp.	128/⩾256	4/128	1/⩾128	0.06/0.5	0.06/0.5	0.06/1	0.12/2
E. faecalis	1/2	2/8	⩾128/⩾128	4/8	4/16	1/32	0.5/16
E. faecium	128/⩾256	⩾256/⩾256	⩾128/⩾128	⩾128/⩾128	⩾128/⩾128	⩾64/⩾64	16/⩾64
Escherichia coli	4/⩾256	1/16	0.06/2	⩽0.03/⩽0.03	⩽0.03/0.06	0.06/16	0.12/16
Klebsiella spp.	4/⩾256	2/64	0.06/16	⩽0.03/0.06	⩽0.03/0.06	0.06/1	0.12/2
M. morganii	32/128	0.25/16	0.25/16	⩽0.03/0.06	0.12/0.5	0.06/1	0.25/4
Proteus mirabilis	1/8	1/8	⩽0.03/0.5	⩽0.03/0.12	0.06/0.25	0.06/1	0.25/2
Proteus vulgaris	4/64	4/64	0.5/16	⩽0.03/0.25	0.12/0.25	0.06/0.25	0.25/2
P. aeruginosa	⩾256/⩾256	⩾256/⩾256	32/⩾128	4/32	1/16	0.5/16	2/16
Serratia spp.	64/⩾256	64/⩾256	0.25/32	⩽0.03/0.25	0.06/0.12	0.25/2	0.5/4
S. aureus	0.25/16	0.25/16	4/⩾128	0.06/8	0.12/16	0.25/16	0.06/4

P1401 Antibacterial effects of marbofloxacin on bacteria isolated from mastitis in cattle

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Mastitis is the inflammation of mammary glands and various microorganisms are known to cause the disease. Many antimicrobial agents have been evaluated to combat the causative agents in order to cure mastitis. As the rate of developing resistance in pathogens may be high against conventional antibiotics, so the efficacy of a new fluoroquinolone antibiotic against pathogens, isolated from clinical mastitis in Shiraz area, was examined in this research.
Milk samples (73) were taken from 28 dairy farms around Shiraz, Iran. Antibiotic sensitivity test (Kirby-Bauer method) was carried out for marbofloxacin, enrofloxacin, gentamicin, penicillin and tetracycline. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of marbofloxacin against the sensitive bacteria was also determined.
Staphylococcus aureus (24.1%), *Escherichia coli* (18.1%), *Streptococcus dysgalactiae* (9.3%), *Corynebacterium* bovis (9.3%), *Staphylococcus epidermidis* (3.7%), and *Pseudomonas aeruginosa* (3.7%), were identified among 54 bacterial isolates. All of the isolated bacteria were sensitive to marbofloxacin, whereas sensitivities against other antibiotics varied from zero to 94.1%. The values obtained as MIC and MBC of marbofloxacin against three bacterial isolates (*Staphylococcus aureus*, *Streptococcus agalactiae*, and *Staphylococcus epidermidis*) were in the range of 0.2 to 1.56 microgram/ml and 0.8 to 6.25 microgram/ml, respectively.
Since the isolated bacteria have shown adequate sensitivities to fluroquinolones, especially marbofloxacin, it is concluded that its usage could be beneficial in the treatment of cows with clinical mastitis.

P1402 In vitro susceptibilities of toxigenic clostridium difficile strains to 10 antimicrobial agents

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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 Immunoassay for Toxins A+B (Immunocard, Meridian Bioscience Inc. Cincinatti Ohio). The susceptibilities to the antimicrobial agents were investigated by E-test (AB Biodisk Solna,

Sweden) according to the manufacturer's recommendations. *B. fragilis* ATCC 25285 was used as quality control strain.
Results: The MICs 50/MICs 90 as well 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, meropenem1/8 (0.125–>32), levofloxacin >32/>32, moxifloxacin 1/>32 (0.75–>32), ofloxacin >32/>32, rifampin ⩽0.002/<0.002 (⩽0.002–>32), tigecycline 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 tigecycline which showed an excellent in vitro activity, will probably offer an alternative therapeutic option for CDAD.

P1403 Comparative in vitro activities of moxifloxacin and 6 other antimicrobial agents against anaerobic bacterial isolates causing intraabdominal infection: results from the PRISMA study

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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: 430 Gram-negative anaerobic bacterial isolates (including *Bacteroides distasonis*, n = 16; *B. fragilis*, 238; *B. ovatus*, 10; *B. thetaiotaomicron*, 79; *B. uniformis*, 33; *B. vulgatus*, 22; and *Prevotella* spp., 21) were collected from 32 centres (including 24 university hospitals) in Germany in 2007. MICs were determined centrally using microbroth dilution for the following antimicrobials commonly used for antianaerobic coverage: ampicillin/sulbactam (AMP/SUL); ertapenem (ERT); meropenem (MER); levofloxacin (LEV); moxifloxacin (MOX); clindamycin (CLI); and metronidazole (MET). EUCAST guidelines were used for interpretation.
Results expressed as MIC50 s and MIC90 s (mg/L) are listed in the Table.

	MIC50/90						
	AMP/SUL	ERT	MER	LEV	MOX	CLI	MET
B. distasonis	4/64	0.12/1	0.25/2	1/32	0.5/16	8/⩾64	2/2
B. fragilis	0.5/4	0.06/1	0.125/2	2/32	0.5/8	1/⩾64	2/4
B. ovatus	0.25/0.5	0.25/0.25	0.25/0.25	8/16	2/4	4/32	2/2
B. thetaiotaomicron	0.25/1	0.25/0.5	0.25/0.5	8/⩾64	2/16	4/⩾64	2/4
B. uniformis	0.25/0.5	0.25/0.25	0.25/0.25	8/32	2/8	4/⩾64	2/4
B. vulgatus	0.25/1	0.06/0.5	0.25/0.25	32/⩾64	16/32	0.12/2	2/2
Prevotella spp.	0.12/0.5	0.06/0.25	0.12/0.25	2/2	0.5/2	0.03/1	1/2

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. vulgatus* with 59% of isolates being resistant). Clindamycin had only poor activity with 20–50% of *Bacteroides* isolates being resistant. Ampicillin/sulbactam and metronidazole were active against most isolates. Ertapenem and meropenem showed the highest in vitro activity against *Bacteroides* species obtained from IAI, however, 8% of *B. fragilis* isolates were resistant to the carbapenems.

Tuberculosis diagnosis

P1404 Accuracy of QuantiFERON-TB Gold test versus Tuberculin Skin test to detect latent tuberculosis infection in HIV-positive individuals in Iran

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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 indurations larger than 5 mm. A whole blood gamma interferon release assay to early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) antigens were performed and γ-IFN were measured via ELISA.
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

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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%, κ0.48, CI (0.36–0.61)) and poor among the vaccinated group (40%, κ0.09, CI (0.03–0.15)). Agreement was high for people with greater degree of exposure to index case, mainly in non-vaccinated group. TST(+)/QFT(−) was the most frequently detected

discordant result, for vaccinated and non-vaccinated groups. A hundred eight non-vaccinated persons showed TST (+), from these 38 were negative for QTF. 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.

P1406 Predictive value of purified protein derivative in pulmonary tuberculosis in a high BCG-vaccinated population

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Objectives: 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 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 tuberculin 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 $\geqslant$ 5 mm had a positive predictive value (PPV) 33% and negative predictive value (NPV) 91%, indurations $\geqslant$10 mm had 34% PPV and 85% NPV and indurations $\geqslant$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. The traditional induration $\geqslant$10 mm does not seem to be the best discriminating value between TST reactions due to infection and those due to vaccination.
The frequencies of TST cut-off diameters will be calculated and presented in the congress.

P1407 QuantiFERON-TB gold cut-off values significance in the diagnosis of active pulmonary *M. tuberculosis* infection

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Objective: Quantiferon-TB Gold (QFT) In-Tube (Cellestis, Australia) is an in vitro diagnostic assay of Interferon-gamma (IFN-γ) detection for the diagnosis of *M. tuberculosis* infection. The purpose of this study was to evaluate QFT diagnostic accuracy by using different cut-off values.
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 tubeculosis. The manufacturer's recommended QFT cut-off value of $\geqslant$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.

P1408 QuantiFERON TB Gold in tube assay for TB diagnosis: a two-year experience

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Objective: To retrospectively evaluate Quantiferon TB Gold in Tube (QFT) test for TB diagnosis in respect to: concordance with TST, as well as, in relation to previous BCG vaccination, to immunnological status, to TB exposure and to active TB.
Materials and Methods: Quantiferon TB Gold in Tube (Cellestis, Australia) was performed according to manufacturers' instructions, in 2000 whole blood samples from an equal number of patients.
Results: In total, QFT(+) results were 627 (31.4%), QFT(–) 1302 (65.1%) and indeterminated 71 (3.5%)
- TST data was available in 1715 cases: in 1087 (63.4%) TST(+), QFT(+) were 461 (42.4%), QFT(–) 611 (56.2%) and indeterminated 15 (1.4%). However, in 628 (36.6%) TST(–), QFT(+) were 100 (15.9%) QFT(–) 487 (77.5%) and indeterminated 41 (6.6%).
- BCG vaccination-TST data was available in 763 cases: 436 (57.1%) were TST(+) QFT(–), 167 (21.9%) were TST(+) QFT(+), 142 (18.6%) TST(–) QFT(–) and 18 (2.4%) TST(–) QFT(+).
- Immunology status data was available in 1554 cases: 243 immuno-suppressed and 1311 immunocompetent. Among the first, TST(+) were 70 (28.8%), QFT(+) 59 (24.3%) TST(–) 173 (71.2%) and QFT(–) 156 (64.2%). In immunosuppressed, indeteminated results were 28 (11.5%) in contrast to 27 (2.0%) in immunocompetent.
- *M. tuberculosis* (MTB) exposure-BCG vaccination-TST data was available in 365 cases: in 234 BCG vaccinated, MTB exposed individuals, QFT(+) were 44 (18.8%) while TST(+) 182 (77.8%)
- Data for sputum culture-QFT test was available in 280 cases: in 74 positively cultured patients, 69 MTB and 5 NTM were isolated. 63 (91.3%) MTB(+) cases were QFT(+) and 1 (20%) NTM (*M. malmoensae*, but with an old MTB history) was QFT (+).

Conclusion: QFT is a very useful method in TB diagnosis because in contrast to TST, it reduces over diagnosis of latent TB, distinguishing truly TB affected among BCG vaccinated, being also, highly correlated to active TB.

P1409 QuantiFERON-TB Gold assay for diagnosis of active tuberculosis

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Objective: The purpose of this study was to evaluate and compare Quantiferon-TB Gold In-Tube (QFT, Cellestis, Australia) and the tuberculine skin test (TST) in patients with active TB, with and without previous BCG vaccination.
Methods: Patients with symptoms compatible with active TB were included. The TST was performed according to the Mantoux method and the QFT assay according to the manufacturer's instructions. The cut-off value for a positive result was $\geqslant$0.35 IU/ml interferon-gamma (IFN-γ). Sensitivity, specificity, positive and negative predictive values

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

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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.

P1411 Evaluation of an oligochromatographic test for identification of mycobacteria most frequently isolated in human from liquid and solid culture media

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Objectives: To evaluate a novel assay (SpeedOligo mycobacteria®, Vircell) for the identification of cultured mycobacteria at the species level.
Methods: A convenience sample of 182 positive cultures for mycobacteria from 121 patients: 22 MGIT® (Becton-Dickinson), 72 Bact/ALERT-MP® (BioMerieux), and 88 LJ. Mycobacteria identification was performed applying Genotype® *Mycobacterium* CM/AS (Hain) and corresponded to 61 *M. tuberculosis* complex, 114 MOTT group-specie identified, 7 *Mycobacterium* sp. identified only to the genus level. Nine mycobacteria-related organisms: 3 *Streptomyces* spp., 4 *Nocardia* spp., 1 *R. equi*, 1 *P. acnes* were included to test the specificity of the assay.
The sample was blindly assayed by the oligochromatographic test. DNA was extracted by heating and centrifugation without requiring a purification step. The 16S rRNA and 16S-23S rRNA gene spacer regions were used as targets for amplification. The amplification products were hybridised on a dipstick using specific probes bound to colloidal gold and to the membrane. The whole procedure took 120 minutes. The results were interpreted by identification of 7 bands specific for each of the following categories: (1) *Mycobacterium* sp. (2) *M. fortuitum*, (3) *M. avium–M. intracellulare*, (4) *M. tuberculosis* complex, (5) *M. kansasii*, (6) *M. gordonae*, (7) *M. abscessus–M. chelonae*.
Results: All 182 positive cultures and 9 mycobacteria-related organisms were tested. No cross reactivity was observed with any mycobacteria-related organisms. The agreement between both tests was 94.5%. SpeedOligo mycobacteria® and reference method identified 61/61 (100%) C.*M. tuberculosis* and 111/121 (91.7%) MOTT: 39 *M. avium*, 15 *M. intracellulare*, 14 *M. gordonae*, 9 *M. abscessus*, 11 *M. chelonae*, 7 *M. fortuitum*, 6 *M. kansasii*, 2 *M. avium* + *M. gordonae*, 1 *M. chelonae* + *M. gordonae*, 7 *Mycobacterium* sp. The discrepancies were distributed in (i) minor: some MOTTs not included in the test that were identified as *Mycobacterium* sp: 1 *M. heckeshornense*, 1 *M. lentiflavum*, 1 *M. scrofulaceum*, 3 *M. simiae*. And (ii) major: 2 *M. marinum* misidentified as *M. kansasii* and 1 *M. scrofulaceum* as *M. avium–M. intracellulare*.
Conclusion: SpeedOligo Mycobacteria® is a fast and sensitive assay for mycobacteria identification. It showed high correlation when results were compared with the standard method. The few discrepancies observed will be resolved by sequenciation and PRA methods.

P1412 TB testing using T-SPOT.TB after overnight sample storage

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Objectives: T-SPOT®.TB is a regulatory approved interferon gamma release assay for the diagnosis of TB infection. The assay procedure currently requires blood samples to be collected and processed within eight hours. However, in some clinical settings it would be advantageous to be able to process samples on the day following blood collection in order to allow overnight shipment of blood samples, for example to central testing laboratories. This study investigated a potential addition to the sample preparation process of the assay that was proposed to allow blood samples to be stored and processed greater than sixteen hours after collection without affecting the clinical result of the T-SPOT.TB assay.
Methods: Blood samples were collected in duplicate from consenting donors at three separate clinical sites; two in South Africa, one in the UK. Anonymised samples were tested with the T-SPOT.TB assay according to the manufacturer's instructions both on the day of blood collection and following overnight storage of the sample at room temperature using the investigational procedure. The assay results were interpreted according to the manufacturer's guidelines and compared between the two assay time points.

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.

	>16 hr +ve	>16 hr −ve	Total
<8 hr +ve	144	5	149
<8 hr −ve	5	198	203
Total	149	203	352

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.

P1413 Test performance of the amplified *Mycobacterium tuberculosis* Direct Test in early diagnosis of Chinese pulmonary tuberculosis

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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 less well studied in Chinese.
Methods: An observational study was conducted to evaluate the test performance of MTD in diagnosis of Chinese PT. All respiratory samples 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. Smears of the sediments were stained by Truant fluorochrom staining method. MCS was done by inoculating sediments into Lowenstein Jensen (LJ) slant and liquid culture medium (Bactec MGIT 960; Becton, Dickinson and Company, Franklin Lakes, NJ). Positive growths of liquid media were sub-cultured into LJ slants. Growth of mycobacteria on LJ slants were subject to molecular detection of specific gene loci (16S rRNA, IS6110, Rv0577) using a polymerase chain reaction-based method to differentiate MTBC from other mycobacteria. 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.

P1414 Laboratory diagnosis of *Mycobacterium xenopi* and its clinical relevance

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Background: Accurate laboratory detection of mycobacterial infection is of utmost importance. Unfortunately, very little is currently known about the performance of automated systems widely used in clinical laboratories.
Methods: Over a period of 3 years (2006–2008), 37 MGIT tubes were scored as negative by the BACTEC™ MGIT 960™ system (Becton-Dickinson). At the end of 6 weeks of incubation they were visually inspected, as recommended by the manufacturer. If positive, an acid-fast stain was performed. Acid fast isolates were then specified using an Hsp65-based PCR assay. 35 of these isolates were identified as *Mycobacterium* Xenopi. These isolates were assessed using Pulse field Gel Electrophoresis (PFGE) and the epidemiological and clinical characteristics of patients with M. Xenopi positive cultures were retrospectively analyzed.
Results: The yearly false-negative detection rate of the automated system studied was 1% as opposed to the <0.5% false negative detection rate mentioned by the manufacturer. During the study period, 35 samples were erroneously diagnosed as negative by the automated system and were finally identified as M. Xenopi positive cultures. Eight cultures out of 17, 7 out 13 and 0 out 10 were isolated from patients hospitalised in one internal medicine department during 2006, 2007 and 2008 respectively. During 2006–7, this department was temporarily re-located due to local refurbishment work, and transferred back to its permanent location in 2008, suggesting that M. Xenopi isolation in this department was dependent upon collection site location. M. Xenopi-positive cultures were mostly of respiratory origin (86%). Patient disease course and clinical features were not consistent with atypical mycobacterial infection in most of the cases.
Conclusions: The false-negative detection rate of the BACTEC MGIT 960 system is higher than previously reported and is mainly accounted for by M. Xenopi cases. The pathogenic relevance of M. Xenopi isolation is questionable.

P1415 Clarithromycin and amikacin versus clarithromycin and moxifloxacin as the treatment for post-acupuncture cutaneous infection due to *Mycobacterium abscessus*: prospective observation study

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Objectives: *Mycobacterium abscessus* (*M. abscessus*) has been a well-documented cause of cutaneous infections following inoculation, minor trauma and surgery. The aim of this study is to compare clarithromycin (CLR) and amikacin (AMK) with clarithromycin and moxifloxacin (MXF) as the treatment for cutaneous infection due to *M. abscessus*.
Methods: An outbreak of postacupuncture cutaneous infection occurred in Ansan, Korea. From Dec 2007 to Mar 2008, about 100 patients developed localised cutaneous infection. *M. abscessus* infection was diagnosed microbiologically or epidemiologically. Patients were treated by combination of CLR (500 mg/day, oral) and AMK (250 mg q 3 times/week, IM) (group I) or CLR and MXF (400 mg/day, oral) (group II). The clinical response of respective treatment groups were evaluated at 8–12 weeks after treatment, completion of treatment, and 2 months after completion of treatment. Treatment success was defined as the absence of symptoms and decrease of more than 50% in subcutaneous nodules or full recovery of skin 2 months after completion of treatment. The efficacy of each treatment groups was assessed by mean duration of medical treatment.
Results: Fifty-two patients were enrolled in this study. Of these, 16 (30.8%) were diagnosed microbiologically. In group I and II, 26 and 16 patients were enrolled, respectively. Other patients were treated

with CLR (n = 7) or MXF (n = 3) alone. There were no significant differences in age (50.5±11.5 vs 51.9±14.7), sex (female) (n = 22 vs 13), co-morbidity, number of nodule (n = 1.9±1.4 vs 2.0±1.6), and pus discharge from wound (n = 8 vs 3) except for initial surgical excision (n = 23 vs 4, p < 0.001). In each groups, 21 (76.9%) and 13 (81.3%) patients completed treatment. 5 (19.2%) and 6 (37.5%) patients required subsequent surgical resection during medical treatment in each groups (p = 0.281). The mean duration (weeks) of medical treatment in group I was significantly longer than that of group II (20.2±3.9 vs 16.4±4.6, p = 0.013). The frequency of drug related adverse events between two groups was not significantly different (n = 14 vs 11, p = 0.518). The most common adverse event was grastrointestinal discomfort.

Conclusion: Combination therapy with CLR and MXF was significantly short in duration of treatment. Our study suggests that combination therapy with CLR and MXF is efficacious for treating *M. abscessus* cutaneous infection.

P1416 Experience with tigecycline in infections due to *Mycobacterium abscessus* complex and *Mycobacterium chelonae*

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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 100 mg 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 (30.8%) 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%), diarrhoea in 12 (23.1%), and asthenia 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

P1417 Serologic response to influenza vaccine in coronary artery disease patients: FLUVAC study

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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 Solmon 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 less 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

	CAD, *n* = 137	HC, *n* = 67
Solomon Islands/3/2006 (H1N1)		
Magnitude of change, × fold, median (IQR)	4 (14)	4 (6)
Serologic response (≥4-fold HI titer rise), *n* (%)	90 (65.7)	39 (58.2)
Wisconsin/67/2005 (H3N2)		
Magnitude of change, × fold, median (IQR)	4 (4)	8 (14)
Serologic response (≥4-fold HI titer rise), *n* (%)	100 (73.0)	50 (74.6)
Malaysia/2506/2004		
Magnitude of change, × fold, median (IQR)	4 (6)	2 (3)
Serologic response (≥4-fold HI titer rise), *n* (%)	78 (56.9)	30 (44.8)

P1418 The efficacy of influenza vaccination in reducing cardiovascular events in patients with coronary artery diseases: IVCAD study

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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 (Flu Vacc) 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 (CAD-influvac, 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

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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 up 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/H3N2 virus, 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.

P1420 Comparison of long-term immunogenicity of influenza vaccine among healthy young adults: intradermal versus intramuscular vaccination

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Objectives: For fear of vaccine shortage during an influenza pandemic, several antigen sparing strategies have been investigated. We are to evaluate the long-term immunogenicity of both one-half dose and one-fifth dose intradermal vaccination compared to the full-dose intramuscular vaccination of a commercial influenza vaccine in healthy young adults.
Methods: During 2006–2007 influenza season, this study was conducted to compare the long-term immunogenicity of intradermal influenza vaccination (one fifth and one half the conventional antigenic contents) with that of intramuscular vaccination (conventional dose) in healthy young adults. Ninety-six subjects were randomly assigned to each group: 0.1 ml dose intradermal vs. 0.25 ml dose intradermal vs. 0.5 ml dose intramuscular vaccination. Serum haemagglutinin inhibition (HI) antibody titers were determined against the recommended influenza strains A/New Caledonia (H1N1), A/Wisconsin/67/2005 (H3N2) and B/Malaysia/2506/2004, at pre-vaccination and 1 and 6 months after vaccination.
Results: Seroprotection (titer±40) rate at 1 month post-vaccination in 0.1 ml dose intradermal, 0.25 ml dose intradermal and 0.5 ml dose intramuscular groups were 76.7% vs. 90% vs. 87.5% for influenza A/H1N1 virus, 70% vs. 93.3% vs. 84.4% for A/H3N2 virus, and 56.7% vs. 60.0% vs. 65.6% for influenza B virus without significant inter-group difference, which were maintained until 6 month post-vaccination. Seroconversion rates were above 40%, while geometric mean titer showed an increase by a factor of >2.5 throughout the 1–6 month periods in all 3 strains irrespective of injection routes and doses. Geometric mean titers of A/H3N2 at 6 month were significantly lower in one-fifth intradermal group compared to the others ($p = 0.03$).
Conclusion: Intradermal administration of one-fifth dose influenza vaccine showed the tendency to elicit lower antibody responses compared to the one-half dose intradermal and conventional dose intramuscular vaccination, but those were still sufficient to meet the requirement guidelines of European Committee for Proprietary Medicinal Products (CPMP) until 6 month time after influenza vaccination.

P1421 Influenza A(H3N2) strains in North Greece: genetic relations and vaccine strain match for the 2004–07 period

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Purpose: The purpose of this study was to identify the A(H3N2) influenza strains that circulated in North Greece during the three influenza seasons 2004–05, 2005–06 and 2006–07, and compare them with the respective vaccine strains.
Methods: A total of 34 A(H3N2) influenza strains from 83 infected individuals of the periods 2004–07 were examined. Regions of the neuraminidase (NA) and haemagglutinin (HA) genes were amplified using one-step RT-PCR and then sequenced. Phylogenetic clades of circulating strains were determined and the HA sequences were compared with those of vaccine strains.
Results: During 2004–05, northern Greek circulating strains were A/Lisbon/3/04-like and A/BW/38/05-like. Both clades had 97% HA sequence homology with the vaccine strain, A/Fujian/411/02. Some of the variations were observed on antigenic sites of the viruses.
During 2005–06, circulating strains were A/Wisconsin/67/05-like and A/Berlin/2/06-like. The first clade had 97%, whereas the second had 98% HA sequence homology with the vaccine strain, A/California/7/04. During 2006–07, Greek strains belonged to three clades. A/Hiroshima/52/05-like and A/Brisbane/10/07-like strains had 99%, whereas A/Nepal/921/06-like strains had 96% HA sequence homology with this year's vaccine strain, A/Wisconsin/67/05. One of the strains that belonged to the last phylogenetic clade, A/Serres/77/07, was chosen by WHO as a

reference strain, because of unique HA sequence variations observed on antigenic sites.
During 2005–06, none of the 11 positive for influenza A(H3N2) patients were vaccinated, whereas during 2006–07, three out of 45 patients were vaccinated. Interestingly, two of these patients were infected by an A/Serres/77/07-like strain.
Conclusions: Results show that two or more clades of influenza A(H3N2) viruses co-circulated during 2004–07 in North Greece. During 2004–05, a relatively low match with the vaccine strain and the altered antigenicity of greek strains, possibly decreased protection offered by the vaccine. During 2005–06, a low match with the vaccine strain was also observed. During 2006–07 the majority of the isolated strains had a high match with the vaccine strain. However, the vaccine did not protect patients infected by A/Serres/77/08-like strains. Constant monitoring of the circulating strains is essential in order to predict the severity of the influenza epidemic and also contribute to the next year's vaccine selection.

P1422 Polymorphism of pertactin gene in circulating *Bordetella pertussis* strains in Serbia

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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 1950 s.
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.

P1423 Is BCG vaccine scar necessary for confirmation of immunity against *Mycobacterium tuberculosis* infection?

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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 children 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 negative, 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.there was no significant statistically distinction between scar positive and negative groups in TST and IGRA results.
Conclusion: 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.

P1424 Meningococcal disease in Italy in the era of conjugate menC vaccination

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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.

P1425 Prospective study assessing the tetanus immunisation status and its determinants among patients consulting for wound care at the emergency department of a non-universitary hospital

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Tetanus is a severe acute toxi-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 universitary 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 unsoecified (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.

P1426 Diphtheria antitoxin levels among adults in Manisa, Turkey

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Objective: Diphtheria is an infectious disease caused by *Corynebacterium diphtheriae*, which can be prevented by immunisation. In pre-vaccination period, disease has engendered high death rates and serious complications among children. In 1930 s, with the vaccination all over the world, a decrease has been determined in morbidity and mortality. In 1990 s, outbreak that occurred in neighbouring countries of Turkey and mostly affecting the adults has attracted the attention to this disease again. The aim of the study was to evaluate the rate of immunity to diphtheria among adults and determining the affected factors.
Method: Blood samples were collected from 856 adults more than 18 years old living in Manisa, Turkey. For each participant, a questionnaire was completed for socio-demographic characteristics and diphtheria immunisation status. Anti diphtheria IgG levels were determined by using enzyme immunoassay method (Genzyme Virotech GmbH, Germany). The levels of 0.1 IU/ml and above were considered to be immune and below the 0.1 IU/ml titers were accepted insufficient immunity.
Results: In the study, it was determined that 61.9% of the people are being protected against the disease whereas 38.1% of them were sensitive. There was a significant relation between the protective immunity and the age. The highest protection rate was existing among people $\leqslant$20 years old and $\geqslant$71 years old. However, the most sensitive age group against the disease was 30–50 ages. There was no significant relation between the sex, alcohol consumption and protective immunity. But, a significant relation between immunity and co-morbidity, living place in childhood, socio-economic level and social status was found.
Conclusion: The high sensitivity rates in adults in our study emphasize the importance of the booster immunisation in adulthood.

P1427 Immunity to pertussis 10 years after acellular booster vaccine in adolescence and response to a second dTpa booster in young adults

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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. This is the first study to evaluate the decennial administration of a dTpa vaccine.
Methods: CMI and antibodies to the three vaccine antigens pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) were 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 young adults 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.

P1428 Evaluation of anti-measles IgG antibody level in medical students 4 years after mass vaccination

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Objectives: Measles is a severe contagious disease that can be prevented by vaccination. In addition to prevalence 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 Zogreb 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$\pm$3/01. History of vaccination in childhood in 194 persons (99%), history of vaccination in 1382 (2004) in 188 persons (95/9%), history of measles infection in 14 persons (7/1%), history of measles in family in 17 persons (8/7%), history of contact with measles case in 19 persons (9/7%) and history of fever in time of vaccination in 1 person (0/5%) was positive. Anti measles IgG antibody in 174 persons (88/8%) was positive, in 19 persons (9/7%) 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.

P1429 Hepatitis B vaccination efficacy among school-age children in south of Iran

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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

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 (HBC 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.

P1430 Immunogenicity of hepatitis A and B vaccines among liver transplant candidates

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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, 41 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

P1431 Food-borne *Salmonella enteritidis* outbreak in a mental health institution

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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 Salmonellae 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 antacid 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 caserean section compared with vaginal birth

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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 $\geqslant$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 0.08% (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

birth. Without post discharge surveillance the infection rate would be underestimated.

P1433 Assessment of the treatment and outcomes associated with severe and mild-to-moderate *Clostridium difficile* infection

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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.

P1434 First results of hospital-based surveillance of *Clostridium difficile*-associated infections in Finnish acute care hospitals

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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 *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 three 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.

P1435 Resource use and costs associated with *Clostridium difficile* diarrhoea in a university hospital

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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 2300 €/patient, but the cost data was highly skewed. Incremental cost for hospital were 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.

P1436 Epidemiology of healthcare-associated *Stenotrophomonas maltophilia* infections in CF and in ICU patients: role of biofilm formation

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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 polystyrene 96-well microtiter plates after 24 h of incubation at 37ºC.
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 epidemic 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 both in 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.

P1437 Creating a collaborative network for the study of bacteraemia in Denmark: frequency of recurrence with the same and different micro-organisms

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Objectives: In most countries bacteraemia is a reportable infection only if the causative agent is subject to national surveillance. In a few countries all cases of bacteraemia 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 bacteraemia. We present data from a newly established collaborative network in Denmark with prospective registration of bacteraemia 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). Bacteraemia 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 $\geqslant$30 days or with another microorganism/s >48 hours after the first positive BC, respectively. We included all patients with bacteraemia during 2006–2007 and follow-up extended for 6 months or until death.
Results: In total, recurrent *Escherichia coli* bacteraemia was the most frequent with 23 to 31 episodes (table). A recurrent episode of either Staphyloccus aureus or *Streptococcus pneumoniae* bacteraemia was rare in the participating hospitals (2.0% to 3.9% and 0.6% to 1.0%, respectively). For all major blood culture pathogens the frequency range was 0% to 4.1% with the exception of *Enterococcus faecalis* (range 0% to 9.4%; table). A recurrent episode with any microorganism was most frequent following bacteraemia caused by *E. faecalis* (range 8.9% to 24.5%) and *Klebsiella pneumoniae* (range 6.7 to 11.3%). The frequency of recurrent candidaemia ranged from 6.9% to 13.6% (table).

Table. Recurrence of bacteraemia within six months of follow-up

Microorganism of the first bacteraemia	Number of first episodes, 2006–2007			Recurrence, same microorganism: n (%)			Any recurrence: n (%)		
	DCM Herlev	DCM Hvidovre	DCM Aalborg	DCM Herlev	DCM Hvidovre	DCM Aalborg	DCM Herlev	DCM Hvidovre	DCM Aalborg
Escherichia coli	740	956	579	31 (4.2)	30 (3.1)	23 (4.0)	83 (11.2)	53 (5.5)	40 (6.9)
Staphylococcus aureus	358	247	234	14 (3.9)	5 (2.0)	7 (3.0)	39 (10.9)	19 (7.7)	23 (9.8)
Klebsiella pneumoniae	151	164	115	6 (4.0)	5 (3.0)	4 (3.5)	17 (11.3)	11 (6.7)	13 (11.3)
Streptococcus pneumoniae	173	191	200	1 (0.6)	2 (1.0)	2 (1.0)	5 (2.9)	11 (5.7)	7 (3.5)
Enterococcus faecalis	140	90	53	8 (5.7)	0 (0)	5 (9.4)	16 (11.4)	8 (8.9)	13 (24.5)
Pseudomonas aeruginosa	79	63	63	1 (1.2)	0 (0)	1 (1.6)	5 (6.3)	5 (7.9)	2 (3.2)
Candida spp.	59	96	58	0 (0)	1 (1.0)	0 (0)	8 (13.6)	7 (7.3)	4 (6.9)
Non-haemolytic streptococci	134	134	61	0 (0)	1 (0.7)	0 (0)	13 (9.7)	5 (3.7)	4 (6.6)

DCM = Department of Clinical Microbiology.

Conclusion: The frequency of recurrent *S. aureus* and *S. pneumoniae* bacteraemia 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 bacteraemia with any microorganism is common subsequent to *K. pneumoniae* and *E. faecalis* bacteraemia 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 bacteraemia management.

P1438 Predictors of mortality in patients with ventilator-associated pneumonia: a meta-analysis

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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.

P1439 Epidemiology of candidaemia and antifungal susceptibility patterns in an Italian medical-surgical intensive care unit between July 2003 and June 2008

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Objective: To evaluate epidemiological trends of candidaemia (CA) and to identify risk factors for it in critically ill patients; to define antimycotic 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, 80% 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-S in only 33% of cases, in 60% SDD and in 7% resistant (R). Most of R or SDD *C. parapsilosis* were isolated during 2005–2006. *C. glabrata* was fluconazole-S in 29% of cases and SDD in 71%. Most of patients had a medical device (i.e. 98% CVC), 96% of them was on antibiotic therapy within the 3 weeks before. 65% underwent surgery, particularly abdominal (79%). CVC-related CA were 59%, mainly due to *C. parapsilosis*. 76% of CA were treated. Fluconazole was employed in 54%, any amphotericin B formulation in 41% and caspofungin in 5%. Treatment was adequate in 74% of CA (inadequacy due to a delay in starting treatment or to posology). 30-days crude mortality was 62.5%, and 61.5% in patients adequately treated (p = 0.27). We didn't find risk factors statistically significant for mortality (table1).

Risk factors	OR	95% CI	*P*
Age (years)			0.7518
<60	1	–	
⩾60	1.300	0.256–6.610	
Gender			0.5995
Female	1	–	
Male	0.727	0.222–2.387	
Underlying disease			0.7435
Other	1	–	
Solid neoplasia	3.000	0.642–14.023	
Acute pancreatitis	3.000	0.423–21.297	
Haematological neoplasia	1.500	0.106–21.312	
Neuropathy	3.000	0.150–59.890	
Cardiopathy + Rheumatic disease + Polytrauma	1.200	0.164–8.799	
Ward of origin			0.3723
Medical	1	–	
Surgical	0.436	0.106–1.804	
Haematology + Emergency + Other hospital	1.067	0.183–6.212	
Diagnosis			0.7155
Post-surgical	1	–	
Respiratory failure	1.143	0.250–5.224	
Septic shock	2.357	0.485–11.452	
Cardiogenic shock + Major trauma + Cardiac arrest	1.714	0.228–12.890	
CVC removed			0.0516
No	1	–	
Yes	0.118	0.014–1.015	
Concomitant bacteraemia			0.1005
No	1	–	
Yes	4.000	0.765–20.918	
Corticosteroids			0.5947
No	1	–	
Yes	1.889	0.181–19.670	
Candida albicans			0.3003
No	1	–	
Yes	2.026	0.532–7.711	

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 frequence of fluconazole-R or SDD *C. parapsilosis* we recommended to start CA treatment with an amphotericin B formulation.

P1440 Epidemiology of candidaemia in non-neutropenic patients in an Italian tertiary-care hospital between 2005 and 2008

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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).

Candida spp.	2005 (n = 37)			2006 (n = 32)			2007 (n = 22)			2008 (n = 9)*			Total
	M	S	UTI	M	S	UTI	M	S	UTI	M	S	UTI	
C. albicans	4	7	11	2	6	8	1	3	8	2	0	1	53%
C. parapsilosis	0	2	7	1	1	3	2	1	2	0	1	1	21%
C. glabrata	1	0	1	0	3	2	0	0	0	1	0	1	9%
C. tropicalis	0	0	2	0	1	2	1	2	1	0	0	0	9%
C. guilliermondii	0	0	0	1	0	1	0	0	0	0	0	0	2%
C. krusei	0	0	1	0	0	0	0	0	0	0	1	0	2%
Others	0	0	1	0	1	0	0	0	1	0	1	0	4%
Total	5, 13.5%	9, 24.3%	23, 62.2%	4, 12.5%	12, 37.5%	16, 50%	4, 18.2%	6, 27.3%	12, 54.5%	3, 33.3%	3, 33.3%	3, 33.3%	100%
C. albicans	22/37 (59.4%)			16/32 (50%)			12/22 (54.4%)			3/9 (33.3%)			
C. non *albicans*	15/37 (40.6%)			16/32 (50%)			10/22 (45.6%)			6/9 (66.7%)			

*First six months.

CVC related CA were 30% and in 9.6% of them CVC was not removed 87% of patients received antifungal therapy and in 92% of cases it was adequate; fluconazole was most frequently employed (74.7%), followed by caspofungin and any amphotericin B formulation (10.3% both), voriconazole (3.4%) and itraconazole (1.2%). No azole-resistant *C. albicans* was isolated, instead of 9.5% of *C. parapsilosis* that was fluconazole-resistant (n 2).

The 30-day crude mortality rate was 26%, 19.7% in adequately treated population.

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

P1441 Infections and mortality of obese patients in the intensive care unit

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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/m^2 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/m^2, 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 infection (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.

P1442 Molecular typing is a cornerstone for infection control in neonatology: a case with extended-spectrum β-lactamase *Escherichia coli* and *Staphylococcus aureus*

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Objectives: Neonatology 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 catalyzed our analysis. Were these infections sporadic or was there a general breech 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, both parents 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.

P1443 Bone biopsy in guiding appropriate antimicrobial usage in chronic osteomyelitis and improving microbiological surveillance of chronic bone infection in orthopaedic surgery

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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 debridment 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 untreating 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.

P1444 Evaluation of device-associated nosocomial infection rates in intensive care units of a university hospital in Turkey

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Objective: To describe the incidence of device-associated nosocomial infections in intensive care units (ICUs) 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.

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 18.1 per 1000 patient days (480/26577). Central venous catheter-associated blood stream infection (CA-BSI) rate was 14.69 per 1000 device days (112/7622), ventilator-associated pneumonia (VAP) rate was 27.79 per 1000 device-days (130/4678) and symptomatic catheter-associated urinary tract infection (CA-UTI) rate was 7.63 per 1000 device-days (138/18083). Device use and incidence densities for specific device-associated infections concerning the types of ICUs are shown in Table I. The most frequent pathogen from patients with BSI was *Staphylococcus aureus* (15.3%) followed by coagulase-negative *Staphylococcus* (CoNS) (14.4%) and *Klebsiella pneumoniae* (8.5%). The most frequent pathogen from patients with VIP was *Acinetobacter* spp (26.3%), followed by *Staphylococcus aureus* (12.9%) and *P. aeruginosa* (6.1%). The most frequent pathogen from patients with CA-UTI was *Candida albicans* (17.6%), followed by *Escherichia coli* (17%) and *Enterococcus faecium* (8.8%).

Of all *Staphylococcus aureus* infections, 87.3% were caused by methicillin-resistant strains. Extended-spectrum β-lactamase rates were 25/40 (62%) for *K. pneumoniae*, 4/7 (%57) for *Klebsiella oxytoca* and (21/49) 43% for *E. coli*.

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 1. Device use and incidence densities for specific device-associated infections according to the types of ICUS

Type of ICU	No. of patients	Patient-days	Device utilisation			Rate per 1000 device days		
			MV	CVC	UC	VAP	CA-BSI	CA-UTI
Anaesthesia	465	2689	0.41	0.47	0.67	29.73	31.45	15.57
Coronary	1161	3430	0.08	0.05	0.40	30.53	0	7.25
Medical	756	3141	0.08	0.18	0.69	0	16.33	12.87
Surgical	1554	6194	0.07	0.44	0.79	28.37	11.78	5.11
Cardiothoracic	692	1801	0.09	0.26	0.65	41.1	1.7	7.51
Respiratory	239	1866	0.38	0.13	0.95	37.97	8.03	3.40
Neurology	187	1156	0.25	0.21	0.98	17.12	8.33	15.90
Neurosurgical	450	3128	0.22	0.39	0.91	37.15	11.45	3.88
Burn	71	1089	0	0.02	0.26	0	0	3.6
Paediatric	362	2083	0.38	0.31	0.31	16.52	13.78	13.82
Total	5937	26577	0.18	0.29	0.68	27.79	14.69	7.63

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.

P1445 Evaluation of the risk factors, mortality and cost of nosocomial infections in intensive care units of a university hospital

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Objective: The aim of this study is to determine the risk factors in nosocomial infections (NIs) in intensive care units (ICUs) and effects of NIs on mortality and cost.

Methods: This study was performed in ICUs of a university hospital between January and June 2007. Nosocomial infections were identified using the CDC National Nosocomial Infections Surveillance system definitions.

Results: The study included 520 patients (7606 patient days). 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 *S. aureus* (22.1%), *Acinetobacter* spp. (18.5%), *Candida* spp. (14%) and *E. coli* (11%). Median lenght of stay of patients with NIs and those without NIs in ICUs were 15.17±10.24 and 7.56±4.69 days, respectively. Extra lenght 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 high APACHE II score, coma, respiratory failure, entubation, steroids, blood transfusion, parenteral nutrition, urinary catheter, haemodialysis, mechanical ventilation, tracheoctomy, central venous catheter, nasogastric tube and the longer stay in ICUs were significantly associated with NIs ($P < 0.05$). Multivariate logistic regression analysis showed that longer stay in ICUs (OR = 5.046; 95% CI, 3.325–7.657; $P < 0.001$), entubation (OR = 4.177; 95% CI, 2.595–6.724; $P = 0.001$), use of urinary catheter (OR = 2.591; 95% CI, 1.133–5.929; $P = 0.024$) and haemodialysis (OR = 2.390; 95% CI, 1.092–5.228; $P = 0.029$) were the independent risk factors. 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: Longer stay in ICUs, use of urinary catheter, haemodialysis and entubation are the independent risk factors for patients with NIs. NI development in ICUs increases the mortality significantly. A patient's acquiring a NI in ICUs costs an extra 3008 Euros.

P1446 Management of infections associated with combat-related extremity injuries in Iraqi patients

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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 Novamber 2008 Sixty three Iraqui patients with compact related ostemylitits 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 recoomonded Clinical Laboratory Standards Institute (CLSI).

Results: From April 2008 to November 2008 Ninety-six specimens obtained from patients hospitalised in Imam Hosein hospital of Mehren city in border of Iran and Iraq. All specimens were sent to Milad Hospital of Tehran. The most important isolated bacteria included: *Staphylococcus* aurues (10 isolates), *E. coli* (7 isolates), *Klebsiella pneumoniae* (5 isolates), *Pseudomonas aeruginosa* (4 isolates), *Proteus mirabilis* (4 isolates), Moganella morgani (3 isolates) and Acintobacter baumannii (2 isolates). All isolates of *S. aureus* were susceptible to vancomycin and 30% were methicillin resistant. All isolates of *E. coli* were ESBLs positive. The most effective antibiotic against ESBLs strains were imipenem. 80% isolates of *K. pneumoniae* also were ESBLs positive. The most effective antibiotic against *P. aeruginosa* and *Acinetobacter baumannii* was colisitin.

Conclusion: Our study showed that *S. aureus* and *E. coli* were the predominant isolates from osteomylitis and all l isolates of *E. coli* and 80 of *K. pneumoniae* were ESBLs positive.

P1447 Long-term trends in the occurrence of adult blood stream infection

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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. Primary 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 comorbid 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.

P1448 Characteristics of infections associated with external drainage devices of cerebrospinal fluid: a retrospective analysis over 12 years

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Objectives: External drainages devices (EDD) are used to treat transitory hydrocephalus. EDD infections often resemble the underlying neurosurgical 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 diagnosed by modified CDC criteria for nosocomial infections. Hospital charts were reviewed to retrieve demographic, clinical, and laboratory data.
Results: 52 patients (median age 55 y, range 20–76 y, 56% males) with EDD infections were included (46 ventricular drainages, 3 lumbar drainages, 2 Jackson-Pratt drains and 1 Liquor guard). Common neurosurgical conditions were subarachnoidal (n = 34), intracerebral (n = 10) and intraventricular bleeding (n = 2). The median EDD-indwelling time was 7 d (range 1–17 d). The reasons for EDD removal were no further indication for draining (n = 37), dysfunction or dislocation (n = 7), patient death and suspicion of infection (each n = 4). Most infections (62%) manifested 3–7 d (median 5 d) after EDD insertion, less often >7 d (27%) or <3 d (12%). In 11 patients (21%), the EDD has been removed up to 10 d before manifestation of infection. The causing pathogens were coagulase-negative staphylococci (n = 20), *Propionibacterium acnes* (n = 4) *Staphylococcus aureus* (n = 3), mixed organisms (n = 10), G+ anaerobe cocci (n = 3) and culture-negative (n = 12). Fever >38ºC was more often present at manifestation of infection than during EDD insertion (77% vs. 12%, $p < 0.001$), whereas other clinical signs and symptoms (headache, vomiting, neck stiffness) were not discriminative for infection. In blood, C-reactive protein was more often elevated (>10 mg/l) at manifestation of infection (88% vs. 55%., $p < 0.01$). In cerebrospinal fluid (CSF), median leukocyte count was higher at manifestation of infection (182 vs. 46 G/l, $p < 0.05$), granulocyte count was similar (46 vs. 15 G/l, $p > 0.05$) and total protein concentration was lower (1060 vs. 2316 mg/l, $p < 0.05$) than during EDD insertion. 8 patients (15%) died because of severe neurological disease.
Conclusions: Most EDD infections manifested 5 days after insertion (range 0–17 d) and in 21% the infection manifested after EDD removal. EDD infections are difficult to distinguish from underlying neurosurgical condition warranting a high index of suspicion and CSF cultures.

P1449 Comparison between multi-locus variable number of tandem repeats and pulse field gel electrophoresis for typing of nosocomial *Pseudomonas aeruginosa* isolates

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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 by 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 thiourea 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) The reproducibility, typeability and discriminatory power (Simpson's Index of Diversity – D) were evaluated and compared for the methods.
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 discriminative.
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*.

P1450 Risk factors for hospital infections with multidrug-resistant bacteria in patients with cancer

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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 methicillin-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 program 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 logistik regression analysis revealed that duration of hospital stay (p = 0.001, OR = 1.045, CI%95 1.018–1.072), solid malinancy (p = 0.029, OR = 3.961, CI%95 1.151–13.631), mechanical ventiation (p = 0.008, OR = 54.804, CI%95 2.858–1050.911), 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 ocurred more frequently in patients with solid malignancy, invaziv procedures, receiving broad spectrum antibiotic and prolonged lenght of hospital stay.

P1451 Dimension of nosocomial infections in a burn care centre: analysis of 7-year active surveillance

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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 age (42.1±9.7), 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. By multiple logistic regression analysis, BSAB (odds ratio (OR: 3.5), comorbidities (OR: 1.8), transfer from another hospital (OR: 2.7), broad spectrum antibiotic usage (OR:2.3) and invasive devices (OR: 1.9) were significantly related to acquisition of NI. No difference was seen in mortality between years, whereas panresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* rates increased after 2004 (P = 0.001). *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* was 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 campaing are required.

P1452 Use of drotrecogin alpha in a surgical septic cohort of patients

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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 fibrinolytic 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 62.41±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% thoracic and 10.3% 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 coadjutants used were: Insulin perfusion (60.3%), corticoids (38.5%) and prophylactic low weigh 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 e 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

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Objectives: The aim of this study was to determinate the incidence, clinical features, microbiologic findings and pronostic 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 (1 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 symtoms was 9.53±6.6 days (R = 2–26). Open craniotomy was achieved in 26.1% and 73.9% closed craniotomy, 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 tracheostomy. The most frequent presenting symptoms and signs were fever (100%), headache (56.5%), altered mental status (52.2%), nausea and vomiting (30.4%) and meningismus (56.5%). The infecting organisms isolated were Coagulase negative sthaphylococcus (11), *Acinetobacter* b. (6), Gram negative bacilli (5), *Candida* parasilopsis (1), Staph. aureus (1). Eighteen cultures were

monomicrobial and 4 polimicrobial. The mean duration of treatment was 29.83±17.1 days (r = 11–30), and intrathecal 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

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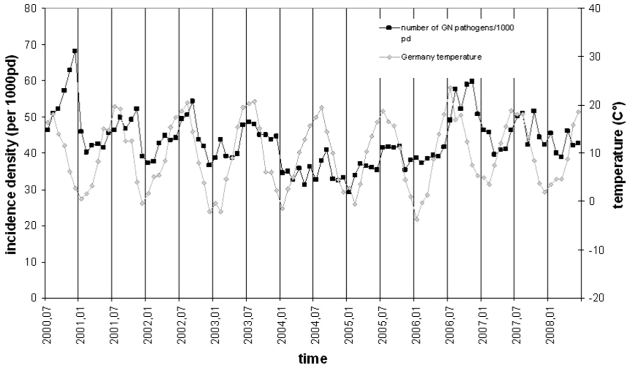
Objective: To look for an association of temperature changes and incidence densities of pathogens in intensive care units participating in SARI (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive Care Units).
Methods: We conducted a prospective unit based surveillance in 49 German 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-positves 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-positve 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

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Objectives: Ceftaroline (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, and 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 1. CPT PK parameters (mean±SD)

Parameter	ESRD		Normal renal function
	dosed before HD	dosed after HD	
C_{max} (μg/mL)	17.5±3.75	29.1±8.49	16.5±3.36
$AUC_{0-\infty}$ (μg·h/mL)	84.1±13.8	128.5±12.5	48.5±9.2
T_{max} (h)	1.01±0.03	1.0±0.0	1.03±0.04
$t_{1/2}$ (h)	6.12±0.81	6.16±0.81	2.75±0.22
CL (mL/h)	4870.71±853.11	3139.87±321.89	8493.66±1550.62

AUC_{0-t} = area under the plasma concentration vs time curve from time 0 to t; $AUC_{0-\infty}$ = area under the plasma concentration vs time curve from time 0 to infinity; CL = plasma clearance; C_{max} = maximum plasma drug concentration; T_{max} = time of maximum plasma drug concentration; $t_{1/2}$ = terminal elimination half-life.

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

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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 NPAG for nonparametric population PK modeling. Monte Carlo simulations were used to predict the time of non-protein bound concentration above the MIC (fT>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 Q12 h (Q8 h) 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 Q12 h were ⩾97.8% against *S. pyogenes* and penicillin-susceptible *S. pneumoniae*. Cefuroxime 250 mg Q12 h (Q8 h) 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 Q8 h instead of Q12 h 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.

P1457 Population pharmacokinetics and optimised dosage regimens of ceftazidime in cystic fibrosis patients and healthy volunteers

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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 ceftazidime in CF-patients has rarely been compared to healthy volunteers in the same study. To compare the population pharmacokinetics and pharmacodynamics of ceftazidime 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 ceftazidime as 5 min intravenous infusion. Ceftazidime 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 ceftazidime dosage regimen 2 g/70 kg WT q8 h 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 ⩽8–12 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 ceftazidime infusion had PTA expectation values between 74% and 94% in CF-patients against *P. aeruginosa* based on susceptibility data from five European CF-clinics.

P1458 Estimation of MIC breakpoints for various intravenous dosage regimens of mecillinam

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Objectives: Mecillinam (i.v.) is used in Denmark for treatment of complicated, even bacteraemic, urinary tract infections (UTI). Apart from being highly active against *E. coli* with MIC values of 0.125–0.5 mg/l, it also retains activity against β-lactamase producing, even many ESBL-producing, *E. coli*, where the MIC has increased to 1–2 mg/l. Recently, the dose vial has been changed to 1000 mg. We therefore wanted to analyze a number of dosage regimens related to the new vial size with respect to relevant MIC levels including ESBL.
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.

P1459 Killing kinetics of cefditoren against recent isolates of *Streptococcus pneumoniae* in Greece

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Objectives: To evaluate the killing kinetic of cefditoren, an oral 3rd generation cephalosporin, against recently isolated *Streptococcus pneumoniae* strains in Greece.
Methods: A total of 262 *S. pneumoniae* strains isolated from invasive and non-invasive infections of adults and children, as well as from nasopharyngeal swabs of preschool healthy children were tested against cefditoren (CFD) by agar dilution. CLSI 2007 methodology was used. Strains with CFD MIC ⩾ 2 mg/L were considered as non-susceptible, according to recent literature. The killing kinetic profile of CFD was explored with killing curves for 20 isolates of *S. pneumoniae* (19 susceptible, 1 non-susceptible, with MICs at 0.125, 0.25, 0.5, 1 and 4 mg/L) using concentrations of 1×, 2× and 4× MIC and within 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h of incubation.
Results: Against the studied isolates, CFD MIC50/90 were 0.03/1 mg/L (range 0.008–4 mg/L). Only 1/262 tested strains (0.4%) exhibited resistance to CFD (MIC = 4 mg/L). Killing curves showed that the bactericidal end point (⩾3 log10 reduction) was reached for 1×MIC concentration within 8 h for 2 strains and within 10 h for 2 strains. For 2×MIC killing was observed within 6 h for 3 strains, within 8 h for 15 strains and within 10 h for 2 strains. For 4×MIC killing was observed within 6 h for 11 strains and within 8 h for 9 strains. Taking into account the average Cmax attained after a single dose of CFD 200 mg in fasted and after fat meal conditions (1.8±0.6 and 3.1±1 mg/L, respectively) and after a single 400 mg dose following fat meal (4.4±0.9 mg/L) a rapid killing effect is anticipated against strains with MIC < 1 mg/L in fasted condition, whereas for strains with MIC ⩾1 mg/L, dosing of 400 mg following a fat meal might be required.
Conclusion: CFD displayed rapid killing (within 10 h) for 1× and 2×MIC concentrations which represented clinically attainable pharmacokinetic targets. CFD could be a useful addition in our community, with favourable resistance profile and killing kinetics.

P1460 Cefuroxime prophylaxis for elective orthopaedic surgery and limits for use of standard dosing

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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-depended 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 for cefuroxime level by HPLC. 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 gender, age, height and weight. From the measured serum concentrations, the values for max concentration, C(0), the volume of distribution, Vd, and the serum half-life, $t_{1/2}$, 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/m^2: 26 (17–44)/29 (21–35).
The calculated values (95% c.i.) for female/male were: Vd in L: 17 (14–19)/19 (17–22); C(0) in mg/L: 114 (94–134)/102 (75–130)/; $t_{1/2}$ in min: 88 (63–112)/81 (63–98).
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 being 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/m^2, but for all our female patients.
Conclusion: For patients with BMI above 33 kg/m^2 and weighting more than 110 kg the cefuroxime dose for orthopaedic prophylaxis may be insufficient.

P1461 Pharmacodynamically-linked variable for the combination of ceftaroline plus Novexel104

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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 ceftaroline (CPT) against a strain of *K. pneumoniae* carrying KPC-2 in high copy number plus TEM-1 and SHV-27 enzymes.
Methods: We employed our hollow fiber infection model (HFIM). CPT was administered as 600 mg Q8 h 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 Q24 h, Q12 h, 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/L 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 3×10^7. All experiments were carried out for $\geqslant$ 10 days.
Results: The MIC for CPT in the presence of 4 mg/L of NXL 104 was 0.75/4 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 Q12 h; AUC 64 Q8 h). 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.

P1462 A pharmacodynamic approach of the antibacterial effect of different β-lactams against mixed inocula of *Haemophilus influenzae* strains and selection of resistant populations

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Objectives: To explore pharmacodynamic activities of cefditoren (CDN), amoxicillin/clavulanic acid (AMC) and cefuroxime (CXM) against an *Haemophilus influenzae* multibacterial niche, as an approach to effects of antibiotics on multiple carriage of *H. influenzae* strains in the nasopharynx.
Methods: Isolates (MICs – mg/l – of CDN, CXM and AMC) used were: One β-lactamase negative (Bneg; 0.015, 1, and 1), one β-lactamase positive (B+; 0.03, 4 and 8), and two strains exhibiting ftsI gene mutations [one β-lactamase negative (BLNAR; 0.015, 8, and 4), one positive (BLPACR; 0.03, 8 and 4)]. A computerised two-compartment pharmacodynamic model (avoiding the dilution of the bacterial inoculum together with the drug along time) was used. Serum free concentrations of antibiotics (A) along 24 h, obtained with 400 mg bid CDN, 500 mg bid CXM and 875/125 mg tid AMC dosing regimens, were simulated in HTM to explore antibacterial activity against initial mixed inocula (4×10^6 cfu/ml) including 25% of each strain (10^6 cfu/ml per strain). Antibiotic-free simulations were used as control (K). Samples for measurement of antibiotic concentrations and colony counts (for the Total population and for each strain) were taken at 0, 2, 4, 6, 8, 10, 12 and 24 h. Areas under killing curves 0–24 h (AUKC; log cfu·h/ml) were calculated. The antibacterial effect over 24 h was measured as the difference between AUKC values in antibiotic-free (AUKC-K) and in antibiotic simulations (AUKC-A): AUKC-K – AUKC-A.
Results: In antibiotic-free simulations, mean AUKC-K ($\log_{10}$ cfu·h/ml) was 150 for the total population, 148 for Bneg, 124 for B+, 108 for BLNAR and 142 for BLPACR strains.
Conclusion: The antibacterial effect (AUKC-K – AUKC-A) was significantly higher ($p < 0.01$) for CDN than for CXM or AMC whether considering Total population, B+, BLNAR or BLPACR strains. CDN at least doubled the antibacterial effect of CXM and AMC against the Total population and those subpopulations exhibiting mutations in the ftsI gene (only CDN exhibited activity against the BLPACR subpopulation).

	Antibacterial effect: AUKC-K – AUKC-A				
	Total	Bneg	Bpos	BLNAR	BLPACR
CDN	70.4±8.8	133.6±7.1	77.8±3.0	66.1±3.7	40.8±7.1
CXM	31.1±2.1[a]	120.3±2.2	53.2±1.3[a]	18.6±3.2[a]	–2.8±2.0[a]
AMC	34.3±1.5[a]	84.4±1.3[a,b]	50.1±1.0[a]	30.4±2.5[a,b]	1.1±1.6[a]

[a]Significant ($p < 0.01$) difference vs. CDN.
[b]Significant ($p < 0.01$) difference AMC vs. CXM.

P1463 Use of the hollow fibre infection model in the pharmacodynamic evaluation of the β-lactamase inhibitor NXL104

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Objective: NXL104 is a novel β-lactamase inhibitor undergoing Phase II clinical evaluation in combination with ceftazidime (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 the study was to determine the importance of NXL104 PK on the PD of CAZ+NXL104 combinations.
Methods: Exponentially growing *Enterobacter cloacae* 293HT96 (Ecl) (AmpC), *Klebsiella pneumoniae* (Kpn) Tunisie C4 (CTX-M-15), Kpn 181 and Kpn 236 (SHV-5, TEM-10) 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 count 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 4 h. Growth of the four strains was fully suppressed throughout the test period following the continuous infusion regimen while the antibacterial effect of the combination was lost when the concentration of NXL104 fell below a critical level as seen after exposure to the human-like profile. Neither CAZ nor NXL104, alone, suppressed the growth of bacteria.
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.

P1464 Activity of moxifloxacin, ceftriaxone and amoxicillin/clavulanic acid against methicillin-susceptible *Staphylococcus aureus* in an in vitro hollow fibre model

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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 HF 1012717, was exposed to MXF (400 mg q.d.: Cmax 2.0 mg/L, T1/2 12 h; MIC = 0.03 mg/L), CRO (2000 mg q.d.: 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 concentrations are equivalent to human free drug serum pharmacokinetics. For the PK/PD model, an initial inoculum of 108 CFU/mL was applied and all experiments were performed, in triplicate, over 24 h. 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 24 h.
Results: 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 5.2 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 24 h.

P1465 Pharmacokinetics of ertapenem in critically ill patients with acute renal failure undergoing extended daily dialysis

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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.

P1466 Increased glomerular filtration rate associated with low imipenem blood concentrations in febrile neutropenic cancer patients

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Objectives: Imipenem (IMP) is a standard therapy in febrile neutropenia. For optimal efficacy in this life-threatening condition, IMP blood concentrations are expected to exceed the minimal inhibitory concentration (MIC) of the causal pathogen throughout the dosing interval. The renal mode of elimination of IMP requires a dose adjustment to the glomerular filtration rate (GFR). Modified pharmacokinetic parameters including GFR variations may influence IMP blood concentrations 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 MIC90 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

(Spearman's coefficient 0.76, $p < 0.001$). IMP trough concentrations were interpreted as low in 24/50 (48%) patients. IMP daily doses and blood concentrations according to the GFR are summarised in the Table. IMP low concentrations were more frequent in patients receiving a daily dose per 100 ml/min GFR <2 g/day when compared with those receiving 2–2.9 or ⩾3 g/day (83, 48 and 0%, respectively, $p < 0.001$).

Conclusion: Increased glomerular filtration rate is frequently observed in febrile neutropenic patients and is associated with insufficient imipenem doses resulting in low blood concentrations. Upfront imipenem dose adjustment to renal function is crucial for optimising antibacterial drug exposure.

Table

	Renal function		
	GFR* <70 ml/min (n = 13)	GFR* 70–100 ml/min (n = 22)	GFR* >100 ml/min (n = 15)
Median IMP dose [g/d]	2 (0.75–2)	2 (2–4)	2 (2–3)
Median IMP dose [g/d] per 100 ml/min GFR	3.3 (2.2–4.5)	2.6 (2.1–4.8)	1.7 (1–2.3)
Median IMP trough [mg/L]	1.5 (0.7–5)	0.6 (0.2–4)	0.3 (0.1–1.2)
Low IMP concentrations (trough < MIC/MIC_{90})	1 (8%)	9 (41%)	11 (73%)

*GFR = Calculated glomerular filtration rate (Cockroft–Gault formula).

Resistance to non-β-lactams in Enterobacteriaceae

P1467 Multiplex PCR for detection of plasmid-mediated quinolone resistance genes in Enterobacteriaceae consecutively isolated from blood cultures

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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 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 qnrA or qepA. Four of 5 qnrB 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*, 8 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.

P1468 Novel plasmid mediated quinolone resistance determinant (Qnr-B21) identified in *E. coli* isolated from hospital effluent

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Objectives: The quinolone group are an important class of broad-spectrum antimicrobial agents. Plasmid mediated quinolone resistance (PMQR) determinants have emerged as a significant concern in recent years. The role the environment plays in the dissemination, maintenance and amplification of antimicrobial resistance is an area of increased interest. This study reports screening of *E. coli* isolates resistant to multiple antimicrobial agents for PMQR determinants and detection of a novel QnrB variant.

Methods: Multi-antimicrobial-resistant *E. coli* were isolated from hospital effluent between March 2006 to June 2007. Antimicrobial susceptibility to 16 antimicrobial agents including nalidixic acid and ciprofloxacin were determined by Clinical Laboratory Standards Institute (CLSI) disk diffusion methods and ciprofloxacin MIC was determined by Etest. Forty three isolates representative of different locations and dates of sampling were screened for the presence of qnrA, qnrB and qnrS using a multiplex PCR assay as previously described. Appropriate control strains were used. Amplicons were sequenced using primers specific to the full coding region of the gene.

Results: One (2%) isolate was positive for qnrB and sequencing revealed it as a novel variant of QnrB differing from the derived amino acid sequence of QnrB8 by a single substitution Asp198Ser and has been provisionally designated QnrB21. This isolate was resistant to ampicillin, nalidixic acid, cefoxitin, trimethoprim, cefpodoxime, cefpodoxime/clavulanic acid, intermediate to tetracycline, cefotaxime.

Conclusion: We describe a novel QnrB variant in a nalidixic acid-resistant but ciprofloxacin susceptible *E. coli* isolate from hospital effluent. Further studies to evaluate the association between the novel QnrB variant and the observed phenotype are under way.

P1469 Plasmid-mediated quinolone resistance in *Salmonella* isolates from different sources of Portugal

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Objectives: Plasmid-mediated quinolone resistance is emerging worldwide in Enterobacteriaceae, including *Salmonella*. Given the importance of fluoroquinolones in therapeutics, we evaluated the incidence of qnr genes and characterised their associated plasmids among a collection of nontyphoidal *Salmonella* isolates with decreased susceptibility to ciprofloxacin (CipR) obtained from different sources in Portugal.

Methods: We studied all CipR isolates (n = 300; MIC 0.25–1 mg/L) obtained from a collection of 1511 Portuguese *Salmonella* isolates (2002–2004). They were identified in human clinical sources (n = 214), food products (n = 82), predominantly poultry (n = 53), and environment (n = 4). Antibiotic susceptibility was determined for streptomycin, kanamycin, gentamicin, ampicillin, nalidixic acid, ciprofloxacin, chloramphenicol, tetracycline, sulfamethoxazole and trimethoprim, by the agar dilution method (CLSI). Qnr resistance genes (qnrA, qnrB and qnrS) were sought by PCR according to Cattoir's method and confirmed by sequencing. Plasmid analysis included conjugation assays, determination of size (S1-PFGE) and content (incompatibility groups by rep-PCR typing method, hybridisation and sequencing). Location of qnr and intI1 genes was done by hybridisation of I-CeuI/S1-PFGE.

Results: Twenty per cent of the isolates of our collection exhibited CipR, being the serotype Enteritidis, isolated from humans and poultry products, the main contributor to this phenotype. None of the isolates was found to be positive for qnrA or qnrB. The qnrS1 gene was detected in a single clinical isolate of the serotype Enteritdis from 2003, which was susceptible to all antimicrobial agents, except ciprofloxacin (1 mg/L), nalidixic acid (32 mg/L) and trimethoprim (>16 mg/L). We identified a conjugative IncN plasmid of ca.30Kb carrying the qnrS1 gene and the intI1 gene. Remarkably, this isolate also harboured a non-conjugative plasmid (50Kb) of the FIIA type which is a common replicon found among *Salmonella* virulence plasmids.

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

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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

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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 (Febuary 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

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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 (IZSA&M), Italy. Molecular analysis was performed by colony hybridisation using specific probes for integrase (IntI) genes, by PCR, sequencing and PFGE analysis. The antibiotics susceptibility profile was determined by Phoenix system.
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 (QRDR) 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 Asp87Asn. Mutations in gyrB where 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.
Conclusion: 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 zoonotically 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

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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 qnr 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.

P1474 Mechanisms of fluoroquinolone resistance in *E. coli* isolates

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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.
Materials and Methods: The study included 114 single *E. coli* isolates from 4 Greek tertiary care hospitals. All strains 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-chlorophenylhydrazone (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 mechanisms was not detected 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. Transferable 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.

P1475 First description of a multidrug-resistant *Citrobacter freundii* carrying both qnrA and qnrB in Aveiro, Portugal

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Objectives: Resistance to quinolone is classically chromosomally mediated. However, plasmid mediated qnr genes emerged, and are now described worldwide. The plasmid encoded nature of these genes may play an important role in dissemination of these determinants in the community and also in the hospital environment.
Methods: *Citrobacter freundii* was identified by the automatic VITEK 2 system and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Étoile, France). Resistance profile was also determined by disc diffusion methods. Presence of ESBL was confirmed by Etest™ (AB Biodisk) ESBL with Cefotaxime/Cefotaxime + Clavulanic acid, Ceftazidime/Ceftazidime + Clavulanic acid and Cefepime/Cefepime + Clavulanic acid strips, according to manufacturer's instructions. PCR, nucleotide sequencing and sequence analysis was employed to identify qnr-encoding sequences.
Results: *C. freundii*, CIT1, was recovered from a 17-year-old boy admitted in mid-August 2008, with the diagnosis of dorso-lumbar spondylodiscitis caused by a MSSA. CIT1 was highly resistant to β-lactams (ampicillin/sulbactam, cefotaxime ceftazidime, cefepime, piperacillin and piperacillin/tazobactam), aminoglycosides (gentamicin and tobramycin) and fluoroquinolones (ciprofloxacin, levofloxacin and norfloxacin); susceptibility was shown only to carbapenems. qnrA and qnrB were detected and were located on different plasmids. qnrA1 was associated with class 1 integron, In37 and a ISCR1. qnrB was not associated either with ISCR1 or ISEcp1, as expected, its genetic environment still being studied. The presence of qnrS and aac(6′)-Ib-cr were not detected.
Conclusion: This is the first report of a *Citrobacter freundii* carrying both qnrA and qnrB. Results obtained also suggest that isolates, which are not commonly linked to sepsis, can be a reservoir of antibiotic resistance genes.

P1476 *Serratia marcescens* carries a chromosomally encoded pentapeptide repeat protein, conferring reduced susceptibility to quinolones in *E. coli*

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Objectives: Since the discovery of qnrA1 in 1998, related plasmid-mediated genes and chromosomal homologues have been described. The presence of peptapeptide repeat proteins (PRPs) with activity against quinolones have been described in the chromosome of several Gram-positive and Gram-negative species. A new pentapeptide repeat protein from clinically relevant specie, *Serratia marcescens*, which confers reduced susceptibility to quinolones when expressed in *E. coli*, is herein reported.
Methods: A protein BLAST analysis revealed the presence of a gene encoding for PRP in *S. marcescens* strain Db11with 80% of amino acid identity with QnrB1. By using the information deposited in the databases, primers suitable for PCR amplification were designed. Fragments carrying the coding region and upstream non coding sequences from several clinical isolates were cloned in pCR-Blunt TOPO. MIC values of quinolones were determined in *E. coli* DH10B and *E. coli* ATCC 25922 using the E test strips. Southern hybridisation was used to explore the presence of this gene within the genus *Serratia*.
Results: Recombinant plasmids coding for PRPs reduced the susceptibility to ciprofloxacin between 8–16 folds in *E. coli* ATCC 25922 and between 3–11 folds in *E. coli* in DH10B, depending on the allele. MIC against nalidixic acid also increases 3–4 folds in the later strain. The sequence upstream from theses genes contains a LexA box involved in SOS response. Results of Southern hybridisation analysis suggest the presence of similar genes in several species within the genus *Serratia*.
Conclusion: The PRP analysed conferred a reduced susceptibility phenotype against fluoroquinolones in *E. coli*. These data provide evidence of its possible role in quinolone resistance in *S. marcescens*. This Gram-negative specie may constitute a reservoir for Qnr-like quinolone resistance protein

P1477 In vivo acquisition of aac(6′)-Ib-cr and mutations in gyrA gene after ciprofloxacin treatment of a clinical qnrS1-positive *Salmonella typhimurium* strain

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Objective: To characterise the phenotypes and genes implicated in the in vivo selection of a clinical *Salmonella typhimurium* isolate with quinolone and aminoglycoside resistance, recovered from a patient after fluoroquinolone treatment.
Methods: A 92-years-old woman was admitted at hospital with acute gastroenteritis and was treated with ciprofloxacin, ampicillin and

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; tet(A)-(E); aph(3′)-Ia, aph(3′)-IIa, aac(6′)-Ib, aadA, 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 assayed.

Results: Both *S. typhimurium* strains presented indistinguishable PFGE patterns and were resistant to tetracycline, streptomycin and sulphonamides, but susceptible to all β-lactams and gentamicin. The MIC values (mg/L) for Se6/Se20 were as follows: nalidixic acid (16/>512), 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, tet(A) 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 tet(A). 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 ciprofloxacin treatment; in vitro transfer of both plasmid-mediated quinolone resistance genes is demonstrated.

P1478 Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum β-lactamase in clinical isolates from Korea

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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, norfloacin, 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.

P1479 A high-level fluoroquinolone-resistant *Salmonella Typhimurium* strain shows decreased invasion ability. Is there any regulatory link?

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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 TolC. 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 TolC 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 operons 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. As 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.

P1480 Decreased cell invasion by a high-level ciprofloxacin-resistant mutant of *Yersinia enterocolitica*

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Objective: The main objective of this study was to characterise a ciprofloxacin-resistant *Yersinia enterocolitica* mutant to determine the potential relationship between quinolone resistance acquisition and cell invasion.

Methods: A quinolone-resistant *Y. enterocolitica* mutant (strain Y-64) was selected in vitro by serial passages from a quinolone-susceptible *Y. enterocolitica* (strain Y-wt) in subinhibitory concentracions of ciprofloxacin. Y-64 was grown in the absence of the antibiotic to obtain a reverted phenotype (strain Y-rev). Sequencing of the quinolone resistance-determining regions (QRDRs) of the gyrA, gyrB, parC and parE genes was performed. The MICs of ciprofloxacin, norfloxacin and nalidixic acid were determined in the presence and absence of PAN (an efflux pump inhibitor). An SDS-PAGE was run with a cell envelope extract to know the outer membrane protein profile. RT-PCR analysis was used to check the mRNA levels of the yadA gene as well as of a homolog of marA (YE1991). The invasion ability was tested with a gentamicin protection assay.

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 fluoroquinolone MICs. AcrAB may only be important for nalidixic acid resistance. This is the first report of a marA homolog found in *Y. enterocolitica* 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.

P1481 Prevalence of hypermutability in clinical isolates of *Klebsiella pneumoniae* and its role in ciprofloxacin resistance

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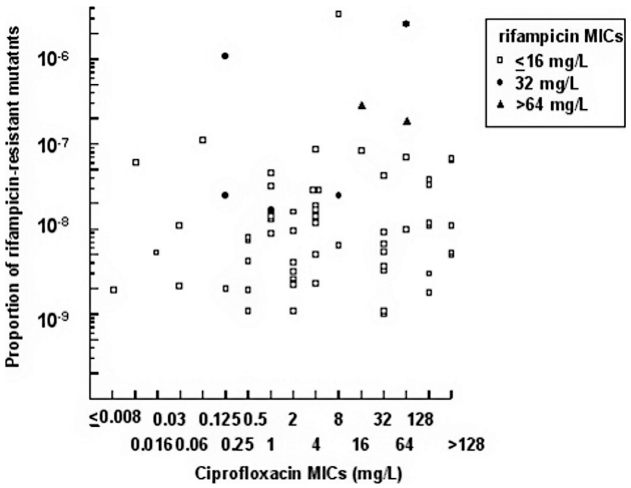
Objectives: To investigate the prevalence of hypermutability in clinical isolates of *Klebsiella pneumoniae*, and its possible role in the accumulation of mutations in the quinolone resistance-determining region (QRDR) and hence in ciprofloxacin resistance.
Methods: Sixty-four distinct clinical isolates 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 or 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.

P1482 Study of fluoroquinolone resistance mechanisms among extended-spectrum β-lactamase-producing *E. coli* urinary isolates in Madrid, Spain

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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.

Group (MIC range, mg/L)	No. of strains	gyrA		parC	
		Ser83	Asp87	Ser80	Glu84
1 (4–8)	5	Leu	Asn	Ile	WT
	2	Leu	Gly	Ile	WT
	1	Leu	Asn	Trp	WT
	1	Leu	Tyr	Ile	WT
	1	Leu	WT	WT	Arg
2 (16–64)	4	Leu	Asn	Ile	WT
	3	Leu	Asn	Ile	Val
	1	Leu	Asn	Ile	Gly
	1	Leu	Asn	Ile	Ala
	1	Leu	Asn	WT	Lys
	1	WT	Asn	Ile	WT
3 (⩾128)	5	Leu	Asn	Ile	Val
	2	Leu	Asn	Ile	WT
	1	Val	Thr	Ile	Val
	1	Leu	Asn	Arg	WT

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 Ser80 Ile, 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%).

P1483 **The differential effect of mutations in RamR, in mediating antibiotic susceptibility in *Klebsiella pneumoniae***

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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 overexpresssing 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-derepression was dependent on the selective agent and secondly to determine which mutations within the ramR gene would impact on resistance profiles upon complementation with wild-type ramR.
Methods: Laboratory mutants from *K. pneumoniae* Ecl8 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.

P1484 **Emergence of AcrAB-mediated tigecycline resistance in a clinical isolate of *Enterobacter cloacae***

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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. Insertional inactivation of the acrB gene in the resistant clinical isolate restored full susceptibility to tigecycline.
Conclusions: *E. cloacae* is a problematic nosocomial pathogen, but is generally susceptible to tigecycline. We report the emergence of low-level resistance to this agent in vivo, 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.

P1485 **BEL-2, an expanded-spectrum β-lactamase with increased activity toward broad-spectrum cephalosporins in *Pseudomonas aeruginosa***

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Objectives: Acquired extended-β-lactamases (ESBLs) are rarely identified in *Pseudomonas aeruginosa*, being of the TEM, SHV, CTX-M, PER, GES, PER, 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. Mating-out assays were performed using *P. aeruginosa* PU21 as reference strain. Cloning of the PCR amplicons 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.

P1486 Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from a French hospital

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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 blaampC 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).

Table. Number of carbapenem resistant/intermediate isolates and corresponding mechanisms involved

	OprD inactivation	AmpC overproduction	ESAC phenotype	Efflux pump overexpression		
				MexAB	MexXY	MexCD
IPM-R (n = 28)	28	24	20	7	2	10
IPM-I (n = 4)	4	1	1	1	0	1

IPM-R: Resistance to imipenem; IMP-I: intermediate susceptibility to imipenem.

78% overexpressed their AmpC β-lactamase. Among those AmpC overproducers, 84% showed reduced MIC values of ceftazidime, cefepime and carbapenems in presence of cloxacillin, suggesting production of an ESAC. The additive presence of ESACs and loss of OprD was observed in 65% of the total collection, those isolates being all resistant to imipenem. Overexpressions of MexAB-OprM, MexXY-OprM or MexCD-OprJ efflux systems were observed in 25%, 6.2% and 34% of the isolates, respectively, suggesting their major contribution in resistance to meropenem observed in 78% of the isolates.
Conclusions: Resistance to imipenem and meropenem in *P. aeruginosa* is multifactorial, combining loss of OprD porin, overexpression of ESACs and efflux pumps overexpression.

P1487 Molecular epidemiology and β-lactamase resistance mechanisms of ceftazidime-resistant *Pseudomonas aeruginosa* causing blood infections in a Brazilian hospital

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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-lactamase (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. Only colistin remained systematically active against all isolates. PFGE analysis identified seven different genotypes. AmpC overproduction was found to be the only mechanism responsible for ceftazidime resistance in four isolates (9.3%). Nine isolates (20.9%) showed an ESBL-positive phenotype, corresponding either to production of ESBL GES-1 (n = 7, 16.3%), or ESBL CTX-M-2 (n = 2, 4.6%). The isolates which were neither AmpC overproducers nor ESBL producers (n = 30, 70%) showed carbapenemase activity. Of those, two isolates (4.6%) produced the ESBL GES-5 (possessing carbapenemase activity), one isolate (2.3%) produced MBL IMP-1, and 27 isolates produced MBL SPM-1 (62.8%). None of the isolates studied co-produced ESBL and MBL determinants. Insertion sequence elements ISCR4 and ISCR1 were associated with blaSPM-1 and blaCTX-M-2 genes, respectively. The blaGES-1, blaGES-5 and blaIMP-1 genes were embedded into class 1 integron structures. All those β-lactamase genes identified here were likely chromosomally-located.
Conclusion: 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).

P1488 Metallo-β-lactamases detection among *Pseudomonas aeruginosa* isolated from bloodstream infections at a university hospital, São Paulo, Brazil

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Objectives: To determine MBL prevalence in *Pseudomonas aeruginosa* (PA) resistant to imipenem; to compare phenotypic and molecular methods to detect MBL; to evaluate the clonality of the MBL producers strains and to determine the susceptibility profile of all isolates included in this study.
Methods: A retrospective study was carried analysing 69 PA strains resistant to imipenem isolated from blood cultures of patients at HC-FMUSP during 2005. They were submitted to the susceptibility profile test by microdilution method and Etest® to colistin. The isolates were also tested for MBL production by phenotypic methods like Double Disk Synergy (DDS), Etest™ MBL, Modified Hodge Test (MHT). The Polimerase Chain Reaction (PCR) was used to detect the following genes: (blaSPM-1, blaIMP-1, blaIMP-2, blaVIM-1 and blaVIM-2). The clonality of the MBL producers was evaluated by Pulsed Field Gel Electrophoresis (PFGE).
Results: Fifty-three isolates (76.8%) had positive results with DDS and Etest® MBL, and 19 isolates (27.5%) were positive by MHT. Twenty-one isolates (30.4%) had a blaMBL gene by PCR, being 17 (81%) positive for blaSPM-1 and 4 (19%) for blaVIM-2. The blaIMP-1, blaIMP-2 and blaVIM-1 genes had not been detected. Mercaptoacetic acid (MAA) inhibitor and MHT showed the best agreement with PCR, with kappa value ranging from 0.81 to 0.86 and 0.79, respectively. Etilenodiaminotetracetic acid (EDTA) inhibitor showed high sensibility (100%), low specificity (33.3%), and poor agreement with PCR. Among isolates producing blaSPM-1 gene, 5 were indistinguishable, 11 were closely related and 1 was possibly related. Among isolates producing blaVIM-2 gene, 2 were indistinguishable, 1 closely related and 1 was different. Among MBL-producing strains, colistin and aztreonam were the most active drugs with 90.5% and 85.7% of sensitivity, respectively.
Conclusions: The results reinforce the Brazilian epidemiology where SPM-1 enzyme is the most prevalent among PA isolates. The DDS method with MAA inhibitor and MHT had the best agreement with PCR, showing themselves as good options for MBL phenotypical detection in microbiology laboratories. The MBL producers had shown multiresistant phenotype with a clonal standard, reinforcing the necessity of infection control practices in our institution.

P1489 Study of metallo-β-lactamase production in clinical isolates of pan-resistant *Pseudomonas aeruginosa*

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Metallo-β-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-disk 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.
Objectives: 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 DDSTT assay employing carbapenem and ceftazidime disk to which EDTA and 2-mercaptopropionic 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 β-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 μg/ml, 256 μg/ml, 32 μg/ml and 256 μg/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. DDSTT using Ceftazidime 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 sensibility 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%, sensibility 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-β-lactamase-producing *Pseudomonas putida* in a Spanish hospital

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Objective: To study the prevalence, nature, involved genetic elements, and the molecular epidemiology of metallo-β-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* PAO1 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/fluorescens* 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-aacA4-blaVIM-2 and intI1-blaVIM-1-aacA4-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

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Background: Although rare, MBL-producing strains of *Pseudomonas* are increasingly reported in Spain. Interpretation of resistance pattern to β-lactams could be difficult when several mechanisms coexist in a strain. Genetic determinants for MBL are located in transposons codifying 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 β-lactams, including cefepime, aztreonam and carbapenems, as well as gentamicin and tobramycin, remaining susceptible only to amikacin and colistin. VIM1-type MBL was determined by sequencing.
Results: The 88.2% of participants answered this QC. Results of the susceptibility studies to β-lactams were almost coincident with that of the reference laboratory, including resistance to carbapenems. Only 67.8% agreement was observed for tobramycin (8% 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 (67.3%) 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 (imipenem+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.

P1492 Relatedness of *Pseudomonas aeruginosa* isolates producers of VIM-2 from two central hospitals in Portugal

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Objective: *Pseudomonas aeruginosa* (PA) remains 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 inter or intra related between hospitals.
Methods: Of the 27 VIM-2 producing isolates, 15 were from Hospitais 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 (WIC), 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 (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.

P1493 Detection of VIM-2 metallo-β-lactamase in *Pseudomonas aeruginosa* isolates from two central hospitals in Portugal

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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.

P1494 Epidemic spread of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-2 metallo-β-lactamase in a Tunis teaching hospital

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Emergence of carbapenem-hydrolysing metallo-β-lactamase (MBL) in *Pseudomonas aeruginosa* has been described worldwide causing a therapeutic problem.
We report an epidemic spread of VIM-2 producing *P. aeruginosa* in a teaching Tunisian hospital.
During the year 2007, 23 carbapenem-resistant *P. aeruginosa* with moderate susceptibility to aztreonam recovered from different wards were analysed for MBL production. MICs of carbapenems were determined by Etest and all isolates were screened for metallo-β-lactamase by EDTA double disc synergy test. Isolates with positive screen test were submitted to PCR analysis with blaIMP, blaVIM and blaVIM-2 primers. The genetic similarity of metallo-β-lactamase positive strains was evaluated by pulsed-field gel electrophoresis
The isolates were particularly recovered from urology (48%), collected from urines (48%) and belonged to serotype O11 (61%). They showed increased MICs of imipenem (MIC range 1–>32 μg/ml; MIC50 = 16 μg/ml) and meropenem (MIC range 1.5–>32 μg/ml; MIC50 >32 μg/ml). The EDTA double disc synergy test was positive for 10 strains (43.5%). Using PCR assay, none of these ten strains was positive for blaIMP gene whereas they were positive for blaVIM-2 gene, Nine out of 10 MBL positive strains corresponded to a single clone (A). The epidemic spread of MBL-producing *P. aeruginosa* isolates, underlines the need of early detection of these resistant strains for the strengthening of hygien measures.

P1495 First identification of metallo-β-lactamase-producing *Pseudomonas aeruginosa* in the Czech Republic

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Objectives: Since 2005, invasive isolates of *Pseudomonas aeruginosa* are collected by National Reference Laboratory for Antibiotics of the National Institute of Health in Prague within the European Antibiotic Resistance Surveillance System (EARSS). During this time, metallo-b-lactamase (MBL) production has been verified by double disk synergy test with EDTA, imipenem and ceftazidime in the strains showing resistance to meropenem (n = 424). First two MBL producing strains of *P. aeruginosa* were identified in the blood cultures from patients with a history of hospitalisation in the Department of Neurosurgery of Masaryk's Hospital (Usti nad Labem, Czech Republic).
Methods: MICs to 12 antibiotics were determined by microdilution broth method according to CLSI recommendation. MICs to meropenem and imipenem were verified by E-test. MBL production was confirmed by spectrophotometric assay. Further characterisation was based on a multiplex PCR, amplification and sequencing of a whole bla gene. Localisation of the gene on class 1 integron was determined by PCR using 5′ CS and 3′ CS and internal primers of bla gene followed by sequencing of amplification products. Strains were typed by PFGE and MLST.
Results: PCR amplification and sequencing revealed the MBL enzyme to be IMP-7 in both isolates carried out on class 1 integron. According to PFGE analysis, both isolates were of the same clone showed a high

degree of the resistance to tested antibiotics. Strains were susceptible only to amikacin (MIC = 8 mg/l) and colistin (MIC = 1 mg/l).
Conclusions: Despite all neighbouring countries, MBLs have never been described and detected in the Czech Republic before this finding. In this time, it is not possible to identify a geographical origin of these isolates. However, the IMP-7 MBL, firstly described in Canada recently in Malaysia and Japan, seems to be uncommon in the Europe. Epidemiological data will be completed by MLST which is on going.
This work was supported by a research project grant MSM 2E08003.

P1496 Multiresistant epidemic clones of *Pseudomonas aeruginosa* in the Czech Republic

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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 set 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 (EARSS) project in 2007. The 108 isolates originated from 49 hospitals in 36 cities. They were tested for susceptibility to piperacillin, ceftazidime, cefepime, 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 while 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 alleles. ST235 and ST175 included 19 and 16 isolates, respectively. The isolates with either ST235 or ST175 originated from 25 hospitals in 19 cities. Each of other eight STs included 3–7 isolates, seven STs were found in 2 isolates and the remaining 24 isolates yielded each a unique ST. Isolates of the same ST had highly similar macrorestriction profiles. 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.

P1497 Isolation and characterisation of an imported pan-resistant *Pseudomonas aeruginosa* clinical isolate producing three different ESBL enzymes, hyperproducing multidrug-efflux pumps

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Objectives: *Pseudomonas aeruginosa* is one of the most frequently isolated nosocomial pathogens, causing life-threatening infections, such as pneumonia, bacteraemia, and wound infections. It exhibits intrinsic resistance to several β-lactams and acquire easily additional resistance mechanisms, including the production of extended spectrum β-lactamases (ESBLs), down-regulation of porins, hyperproducing multidrug efflux-pumps, confer resistance to almost all antipseudomonas antibiotics.
Methods: During the period of 2004 to 2008 we isolated 27 ceftazidime resistant, non-mucoid *P. aeruginosa* isolates from different non-cystic fibrosis patients, hospitalised in nine different hospital wards of South-Hungary. Identification by VITEK 2 system and susceptibility test by disk-diffusion method was performed, using CLSI breakpoints. The genes of the extended spectrum β-lactamases (PER-1, PER-2, TEM, SHV, GES, VEB-1, OXA groups) were looked for by PCR methods. The iso-electric focusing of the β-lactamases was performed, the enzymes were visualised with nitrocefin. To investigate the location of the β-lactamase gene plasmid purification, PCR detection of the Tn1213 specific IS element were performed. The transcription of the chromosomal genes encoding the OprD, ampC, and the efflux pumps MexAB-OprM, MexXY-OprM, MexCD-OprJ was studied with real-time PCR assays. The genetic relatedness of the strains was investigated by PFGE and MLST analyses.
Results: PCR experiments revealed the presence of blaPER, blaOXA-I, II group in one isolate. Sequencing of the coding region and the RFLP analyses identified the PER-1, OXA-2 and OXA-74 genes. The real-time PCR assays revealed, that this strain hyperproduces two different multidrug efflux-pumps, namely the MexAB-OprM and the MexXY-OprM. According to the MLST typing analyses, this strain belongs to a clonal complex, previously identified in VIM metallo-β-lactamase producers in Hungary, namely CC11. Interestingly, the pan-resistant strain was isolated from a polytraumatised Romanian citizen on admission to the hospital of Szeged. This suggests the possibility, that this strain was imported to Hungary from abroad.

P1498 Emergence and persistence of multidrug-resistant *Pseudomonas aeruginosa* serogroups O11 and O12

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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 serogroups in the light of the global *P. aeruginosa* population structure.
Methods: 328 unrelated *P. aeruginosa* clinical CF (43) and non-CF (142), environmental (63) and animal (55) strains, including 61 serotype O11 and 24 O12 strains, collected between 1882 and 2008 in 69 localities (30 countries, 5 continents) were characterised by fingerprinting (FAFLP), MLST (oprI, oprL and oprD genes), pyoverdine receptor gene typing, prevalence of exoS and exoU genes and serotyping. 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 no 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).
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**

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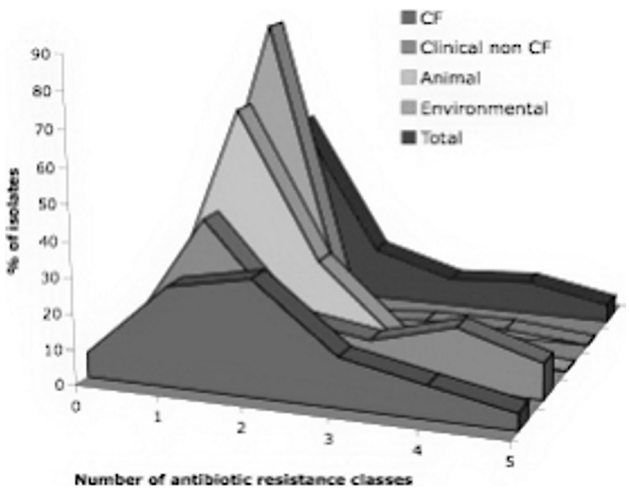
Objectives: *P. aeruginosa* exhibits high inherent antibiotic resistance (ABR) combined with acquired ABR mechanisms. Important acquired ABR mechanisms in *P. aeruginosa* are β-lactamases (BL) and aminoglycoside-modifying enzymes (AME). The objective of this study was to analyze the relation between 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**

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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 attendance. 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%), 52.9% (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 ciprofloxacin, 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**

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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 SpeI 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

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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 cut-off, 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 (cefepime-resistant isolates were also often resistant to other antibiotics [GEN, 24%; AMK; 8%; ATM, 27%; MEM, 20%; and CIP, 20%]).

	Global		H1 (n = 12)		H2 (n = 30)		H3 (n = 18)		H4 (n = 22)		H5 (n = 56)	
	MIC_{50}/90	I/R (%)[a]	MIC_{50}/90	I/R (%)[a]	MIC_{50}/90	I/R (%)[a]	MIC_{50}/90	I/R (%)[a]	MIC_{50}/90	I/R (%)[a]	MIC_{50}/90	I/R (%)[a]
GEN	**2/64**	**0.0/25**	**2/64**	**0.0/25**	**2/64**	**0.0/30**	**4/64**	**0.0/33**	**2/32**	**0.0/27**	2/8	0.0/20
AMK	4/16	9.4/8.0	4/8	0.0/8.3	4/32	13/13	8/16	11/5.6	4/32	18/14	8/8	3.6/5.4
MEM	1/16	0.0/10	1/4	25/8.3	**2/16**	**6.7/37**	1/4	17/5.6	**1.0/16**	**4.5/27**	**1/16**	**16/27**
FEP	**8/64**	**0.0/46**	**4/32**	**0.0/33**	**16/64**	**0.0/70**	**12/64**	**0.0/44**	**6/64**	**0.0/36**	**8/64**	**0.0/37**
CIP	**0.25/8**	**7.2/22**	**0.25/8**	**8.3/25**	**0.4/16**	**6.7/37**	**0.25/16**	**11/33**	0.19/8	0.0/14	0.19/16	8.9/16.1
TIC	**64/512**	**0.0/87.0**	**32/256**	**0.0/78**	**128/256**	**0.0/83**	**64/256**	**0.0/80**	**48/512**	**0.0/91**	**64/512**	**0.0/90**
ATM	**12/32**	**67/30**	**6.0/32**	**67/33**	**16/32**	**67/33**	**16/32**	**56/33**	**16/128**	**64/36**	**8/32**	**75/23**

[a] EUCAST breakpoints: gentamicin (GEN): S⩽4, R>4; amikacin (AMK): S⩽8, R>16; meropenem (MEM): S⩽2, R>8; cefepime (FEP): S⩽8, R>8; ciprofloxacin (CIP): S⩽0.5, R>1; ticarcillin (TIC): S⩽16, R>16: aztreonam [ATM]: S⩽1, R>16.
Figures in bold indicate situations (global or per hospital) in which resistance to a clinically-used given antibiotic exceeds 20% of isolates.

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

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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 evaluable 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%), ceftazidime (68.9–77.1%), and tobramicin (64.6–70.9%). On the other hand, gentamicin (55.1–63.5% of tested strains), aztreonam (57.5–66.8%), ciprofloxacin (55.7–65.0%), 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 tobramicin ($p < 0.04$).
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, carbapanems, piperacillin-tazobactam, amikacin, imipenem, and ceftazidime 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* strains, remain as a possible component of combined antimimicrobial strategies, when multiresistant pathogens are of concern.

P1504 Incidence of *Pseudomonas aeruginosa* resistant to colistin in a tertiary care hospital

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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 with 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

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 coinfected with *A. baumannii*, 2 with extended-spectrum β-lactamase producing *Escherichia coli* and 2 with methicillin-resistant *Staphylococcus aureus*. Only the four patients coinfected 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.

P1505 Resistance and epidemiology of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients: a French multi-centre study

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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 cloxacillin 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 strains were genotyped by MLVA (Multiple Loci Variable Number of Tandem Repeat Analysis) using 15 Variable Number Tandem Repeat (VNTR) markers. MLVA clustering analysis was performed using the categorical coefficient and the Unweight Pair Group Method with Arithmetic mean (UPGMA).
Results: The resistance rates of *P. aeruginosa* (according to the EUCAST breakpoints) were found to increase with patients' age for all the antibiotics tested except for colistin. 128/204 strains (62.7%) displayed a wild-type susceptibility phenotype to β-lactams. Susceptibility rates to ticarcillin, piperacillin, piperacillin-tazobactam, ceftazidime and imipenem were 67%, 74%, 77%, 81%, and 80%, respectively. Analysis of the resistance mechanisms to β-lactams in 67 isolates with ticarcillin MIC >16 mg/L showed that 39/67 strains overexpressed β-lactamase AmpC, 57/67 overproduced efflux pumps MexAB-OprM and/or MexXY/OprM while 62/67 lacked carbapenem-specific porin OprD. 11/67 strains were found to harbour acquired β-lactamases including VIM (n = 1), TEM-2 (n = 1), PSE-1 (n = 1), as well as OXA-1-like (n = 4) and OXA-2-like derivatives (n = 4). MLVA analysis identified 111 different genotypes and 41 clusters among the 204 collected isolates, three of which correspond to previously identified strains (clone C, sequenced strain Pa 14, sequenced strain 3719).
Conclusion: The resistance of CF strains of *P. aeruginosa* to β-lactams is quite high in France but not really different from that reported for nosocomial strains. It mainly results from intrinsic mechanims emerging in individual strains submitted to repeated courses of chemotherapy.

P1506 RT-PCR to investigate the roles of efflux, porins and β-lactamases in multi-resistance of *Pseudomonas aeruginosa* from cystic fibrosis

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Introduction: *P. aeruginosa* isolates from cystic fibrosis (CF) patients are often highly resistant to multiple antimicrobial classes, with phenotypes that do not match those for single efflux, β-lactamase or impermeability mechanisms. We investigated whether real-time reverse transcriptase (RT)-PCR could be used to identify the combinations of mechanisms present.
Methods: 25 *P. aeruginosa* isolates were studied, collected from UK CF patients in 2007–8; 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 PAO1. 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. Transcription 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.
Results: The 25 isolates included representatives resistant to carbapenems (n = 20), ceftazidime (13), aztreonam (16), ciprofloxacin (21), and aminoglycosides (20). In univariate analysis, overexpression of mexA and mexC was significantly associated with resistance to meropenem, cephalosporins, aztreonam and carbenicillin;mexX overexpression correlated with elevated amikacin MICs, and mexE overexpression with raised cefotaxime MICs. Increased ampC transcription was associated with resistance to cephalosporins and, surprisingly, meropenem, while reduced oprD expression was associated with carbapenem resistance and presumably owing to combination with other mechanisms, not causality with raised MICs of aminoglycosides and ciprofloxacin. Logistic regression identified reduced oprD expression ($P = 0.048$) and mexA overexpression ($P = 0.057$) as the main mechanisms underlying meropenem resistance, whilst mexA overexpression was also associated with resistance to aztreonam ($P = 0.023$). EDTA synergy suggested carbapenemase activity in 2 isolates, but blaMBL genes were not detected by PCR.
Conclusions: RT-PCR proved useful for investigating the multiplicity of mechanisms present in these complex *P. aeruginosa* isolates from CF.

P1507 β-lactamase activity evolution of *Aeromonas hydrophila* CphA metallo-β-lactamase by design of a chimeric CphA-VIM-1 enzyme

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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, 25kDa, pI 8.0) and VIM-1 (266 aa, 26kDa, pI 5.2) contain α-β-β-α 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

as template. blaCphA-VIM-1 gene was cloned into pBC-SK vector and the recombinant strain was inserted by transformation into *E. coli* JM109. The determination of MICs was performed by the conventional macrodilution broth procedure as recommended by CLSI.
Results: The CphA-VIM-1 chimera was made using CphA enzyme as scaffold. A domain of 92 amino acid residues, including Asn116, Asn118 and Asp120, was substituted in CphA metallo-β-lactamase by the corresponded domain of 78 amino acid from VIM-1. In vitro susceptibility was tested on *E. coli* pBC-CphA-VIM-1, *E. coli* pBC-CphA and *E. coli* pBC-VIM-1 versus a large pattern of antibiotics. *E. coli* pBC-CphA-VIM-1 recombinant strain showed an higher MIC value for piperacillin (MIC value, 32 mg/L) with respect to *E. coli* pBC-CphA (8 mg/L) and *E. coli* pBC-VIM-1 (8 mg/L). Concerning cephalosporins, MIC values for cefazolin was 64 mg/L for *E. coli* pBCCphA-VIM and 2 mg/L for *E. coli* pBC-CphA and *E. coli* pBC-VIM-1. The molecular weight and pI was evaluated on pure enzyme by SDS-Page and isoelectrofocusing and seem to be 24 kDa and 6.2, respectively.
Conclusion: Starting with CphA enzyme, that hydrolyzes only carbapenems, we have obtained a new chimeric enzyme that evolves versus a much broader spectrum activity.

Integron-related antibiotic resistance

P1508 Prevalence of class 1 and class 2 integrons among *Escherichia coli* isolates of human and animal origin in Lithuania

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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. aadA1, estX+aadA1, dfrA14+aadA6, aacA4+catB3+dfrA1+orfX, 330 bp+sat2+aadA2 gene cassette arrays were exclusively associated with class 1 integrons in isolates from animal origin, whereas dfrA5+ereA2, oxa30+aadA1 and dfrA17+cmlA 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.

P1509 The genetic environment influencing selection and persistence of sul2 in clinical isolates

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Objectives: sul2 has been found as the most prevalent sulfonamide resistance gene in isolates from distinct origins and species. Firstly identified on plasmid RSF1010 in 1988, it has been frequently found adjacent to strAB and ISCR2 sequences on different plasmids. In this work, we determine sul2 genetic environment in human enterobacterial clinical isolates.
Methods: A collection of 129 clinical enterobacterial isolates containing sul2 from our institution (1988–2006) including extended spectrum- (ESBL, n = 96)/metalo- (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 (5–12Kb). Most conjugative plasmids belonged to rep type Inc B/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-*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.

P1510 Sul3-carrying class 1 integrons in clinical Enterobacteriaceae isolates and their plasmid association

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Objectives: 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 or metallo-β-lactamases (ESBL or MBL) producers (n = 241) and non producers (n = 103) was studied. Clonal relatedness was established (PFGE, phylogenetic groups) and antibiotic susceptibility determined by disk diffusion (CLSI). Characterisation of sul3-integrons was determined by long-PCR, RFLP and sequencing. Plasmid analysis included size determination (S1 nuclease), and incompatibility grouping (PCR of replicases and relaxases, hybridisation, sequencing).
Results: We identified 22 sul3-positive *Escherichia coli* isolates (6.4%) from 1997 to 2006. They mainly belonged to A and B1 phylogroups (n = 14/22). sul3-positive isolates showed decreased susceptibility to sulfonamides (86%), streptomycin (86%), trimethoprim (77%), tetracycline (77%) and chloramphenicol (68%). Most of the isolates also contained sul1 and sul2 and all were ESBLs (n = 22) or MBL (n = 1) producers and mainly recovered from community patients. sul3 was transferable in 86% of the cases. Five different integron arrays

were found, three of them identical to those previously described. All of them showed a common region qacH-IS440-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

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Objectives: 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 tranposons 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 (In) 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, V, 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 promotor 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 recombinatorial events between mobile elements widely disseminated among human Enterobacteriaceae isolates.

P1512 Antimicrobial resistance of *Salmonella* strains implicated in 38 community outbreaks in northern Spain

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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 (PHLPA), the other 28 isolates were collected from foods outbreak associated in the PHLPA. 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 clase 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 multirresistant, each with a R-profile: Ap, Te, Su, Cm, S (carrying a class 1 integron, InH) 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 Kb), Typhymurium (90 and 125 kb, this is an 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.

P1513 Occurrence and characterisation of class 1 integrons from isolates collected in a hospital's sanitary facility in Aveiro, Portugal

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Objective: In a hospital environment, antibiotic resistant strains are not only confined to the patients but they can also "colonise" the surrounding environment. We investigated the presence of antibiotic resistance mobile elements, such as class 1 integrons, in bacterial strains collected in a hospital's female ward sanitary facility (SF).
Methods: Sterile swabs were rubbed over various SF surfaces and incubated in rich medium at 37ºC. Serial dilutions were plated in MacConkey agar for Gram negative isolates selection. Phenotypically different colonies were selected and their clonal relationship was evaluated by rep-PCR. Identification to the species level and antibiotic susceptibilities were determined using automatic VITEK2 system and Advanced Expert System (VITEK2 AES) (BioMérieux, Marcy L'Étoile, France). Presence and characterisation of Class 1 integrons was performed by PCR followed by nucleotide sequencing. Nucleotide and deduced aminoacid sequences were analyzed with Blast and ClustalW programs.
Results: Eighty-two genetically different strains were identified belonging to the following genera: *Acinetobacter*, *Alcaligenes*, *Citrobacter*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Morganella*, *Pseudomonas*, *Proteus*, *Serratia* and *Stenotrophomonas*. Class 1 integrons were amplified from 24% of the isolates. 17 different gene arrays were identified: 35% consisted of novel combinations of gene cassettes and 41% consisted of a first description in the species. aacA4 was the most abundant gene, followed by aadA1 and aadA2. 67% (n = 18) of *Pseudomonas* spp. integrons possessed a metallo-β-lactamase gene (blaIMP-8 or blaVIM-2) which were found exclusively in this genus. dhfr, blaPSE-1, arr-3, catB3, ORF and tnpA genes were also detected, but at lower rates. Ancestral integrons possessing the tniC-gene instead of the traditionally 3′CS were identified in 2 isolates (*P. aeruginosa* and *P. putida*).
Conclusions: The results showed that class 1 integrons are widely spread among the isolates collected from SF, particularly among opportunistic strains. Several new gene arrays were characterised. This is the first description of tniC-containing integron in Portugal. These results suggest that strains harbouring class 1 integrons constitute part of the normal human flora, able to survive outside a host, constituting a vehicle to the dissemination of bacterial strains between patients.

P1514 Characterisation of class 1 and class 2 integrons among bacteria isolated from an urban waste water treatment plant in Italy

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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).

Organisms	Integrase type (location)*	Variable region size (bp)	Variable region content	Resistance phenotype	Plasmid	β-lactamase
Escherichia coli	I (C)	1664	*dfr17, aadA5*	AMP, AMX, AMC, STX	+	bla_{TEM}
Escherichia coli	II (P)	1227	*dfr1, sat1*	AMP, AMX, PIP, CAZ, STX	+	–
Serratia liquefaciens	I (C)	–	ND	AMP, AMX, PIP, CAZ	+	–
Enterobacter agglomerans	I (C)	–	ND	AMP, AMC, AMX, CEF, CAZ	–	–
Acinetobacter baumannii	I (C)	1664	*dfr17, aadA5*	AMP, AMX, PIP, CEF, CTX, CAZ, IMP, STX	+	–
Serratia odorifera	II (C)	–	ND	AMP, AMX, PIP, CEF	+	–
Escherichia coli	I (C)	880	*aadA10*	AMP, AMX, AMC, PIP, LVF, STX	+	bla_{TEM}
Acinetobacter baumannii	I (C)	–	ND	AMP, AMX, AMC, PIP, CEF, CTX, CAZ, LVF	–	–
Escherichia coli	II (C)	2158	*dfr1, sat1, aadA1*	AMP, AMX, AMC, PIP, CEF, CTX, STX, LVF	+	bla_{TEM}
Escherichia coli	I (C)	–	ND	AMP, AMX, AMC, PIP, CEF, CAZ, LVF	–	bla_{TEM}
Escherichia coli	I (C)	–	ND	AMP, AMX, PIP, CEF, CAZ, STX	–	bla_{TEM}
Klebsiella pneumoniae	I (C)	1664	*dfr17, aadA5*	AMP, AMX, AMC, CEF, STX, LVF	+	bla_{TEM}
Klebsiella oxytoca	II (C)	2158	*dfr1, sat1, aadA1*	AMP, AMX, PIP, TZP, CEF, STX	+	bla_{TEM}
Aeromonas hydrophila	I (C)	–	ND	AMP, AMX, CEF	–	–
Escherichia coli	I (C)	–	ND	AMP, AMX, PIP, CEF, CAZ	–	bla_{TEM}
Escherichia coli	I (C)	1664	*dfr17, aadA5*	AMP, AMX, AMC, PIP, CEF, STX	–	bla_{TEM}
Citrobacter freundii	I (C)	880	*aadA10*	AMP, AMX, AMC, PIP, CEF, STX	–	–
Klebsiella oxytoca	I (C)	1664	*dfr17, aadA5*	AMP, AMX, PIP, CEF, CTX, CAZ, LVF, STX	+	bla_{TEM}
Acinetobacter baumannii	I (C)	1664	*dfr17, aadA5*	AMP, AMX, PIP, CEF, CTX, CAZ, LVF, STX	+	bla_{TEM}
Escherichia coli	I (C)	–	ND	AMP, AMX, PIP, CEF, LVF	+	bla_{TEM}
Klebsiella pneumoniae	I (C)	1664	*dfr17, aadA5*	AMP, AMX, AMC, PIP, CEF, STX, LVF	+	bla_{TEM}
Citrobacter freundii	II (P)	2158	*dfr1, sat, aadA1*	AMP, AMX, AMC, PIP, TZP, CAZ, IMP, STX	–	–
Enterobacter agglomerans	I (C)	–	ND	AMP, AMX, CEF, CAZ, STX	–	–
Enterobacter agglomerans	II (C)	2158	*dfr1, sat, aadA1*	AMP, AMX, AMC, PIP, CEF, CAZ, IMP, STX	–	bla_{TEM}
Pseudomonas putida	I (C)	930	*blaVIM-1*	AMP, AMX, AMC, PIP, CEF, CTX, CAZ, IMP	–	bla_{VM}
Pseudomonas fluorescens	I (C)	1281	*blaIMP-22, ORFXX*	AMP, AMX, AMC, PIP, CEF, CTX, CAZ, IMP	–	bla_{IMP}
Pseudomonas fluorescens	I (C)	1910	*blaIMP-22, ORFXX, aacA4*	AMP, AMX, AMC, PIP, CEF, CTX, CAZ, IMP, STX	–	bla_{IMP}
Enterobacter intermedius	I (C)	1664	*dfr17, aadA5*	AMP, AMX, PIP, CEF, CAZ, STX	–	bla_{AmpC}
Escherichia coli	I (C)	570	*mobA*	AMP, AMX, AMC, PIP, CEF, CTX, CAZ, STX	+	bla_{TEM}
Escherichia coli	I (C)	–	ND	AMP, AMX, PIP, CEF	–	bla_{TEM}
Alcaligenes faecalis	I (C)	–	ND	CEF, CTX, CAZ	+	bla_{CTX-M}
Escherichia coli	I (C)	880	*aadA10*	AMP, AMX, PIP, CEF, CAZ, STX	–	bla_{AmpC}

*C: chromosomal localisation; P: plasmidic localisation. ND: not detected.

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 6 mg/L and imipenem 2 mg/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 β-lactamase genes, such as blaTEM-1 gene, blaCTX-M-1-type gene (detected in *Alcaligenes faecalis*), blaIMP-22, blaVIM-1 and blaAmpC 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*

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Objective: The main objective of this study was to determinate 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-carbapenamase production was also investigated by PCR amplification and sequencing of blaIMP, blaVIM and blaSPM 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 blaSPM-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.

Table 1. Comparison between antimicrobial susceptibility rates of *P. aeruginosa* with and without *intI1* and of *Acinetobacter* spp. with and without *intI1* and *intI2*

Antimicrobial[a]	Susceptibility (%)								
	P. aeruginosa (n = 149)			*Acinetobacter* spp. (n = 63)					
	intI1 + (n = 68)	*intI1* − (n = 81)	P value[b]	*intI1* + (n = 11)	*intI1* − (n = 52)	P value[b]	*intI2* + (n = 23)	*intI2* − (n = 40)	P value[b]
AMI	191	96.3	<0.0001	0	28.9	0.0528	0	37.5	0.0004
GN	1.5	93.8	<0.0001	0	26.9	0.1034	0	35	0.0010
TOB	1.5	95.1	<0.0001	0	59.6	0.0003	21.7	65	0.0015
CIP	2.9	70.4	<0.0001	0	23.1	0.1044	0	30	0.0024
NOR	2.9	72.8	<0.0001	–	–	–	–	–	–
LEV	2.9	66.7	<0.0001	0	23.1	0.1044	0	30	0.0024
CAZ	33.8	77.8	<0.0001	0	26.9	0.1034	0	35	0.0010
CTX	–	–	–	0	0	ND	0	0	ND
CFP	4.4	71.6	<0.0001	–	–	–	–	–	–
FEP	27.9	82.7	<0.0001	0	25	0.1005	0	32.5	0.0023
SAM	–	–	–	100	90.4	0.5763	100	87.5	0.1488
AZT	19.1	64.2	<0.0001	–	–	–	–	–	–
TZP	30.9	79	<0.0001	0	25	0.1005	0	32.5	0.0023
TIM	7.4	71.6	<0.0001	0	19.2	0.1868	0	25	0.0099
IMP	25	92.6	<0.0001	100	100	ND	100	100	ND
MER	20.6	92.6	<0.0001	72.7	92.3	0.1314	100	82.5	0.0414
PB	100	100	ND	100	100	ND	100	100	ND
CT	–	–	–	100	100	ND	100	100	ND
DO	–	–	–	100	100	ND	100	100	ND
MH	–	–	–	100	100	ND	100	100	ND
TGC	–	–	–	9.1	61.5	0.0020	73.9	40	0.0175

[a] AMI, amikacin; GN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; NOR, norfloxacin; LEV, levofloxacin; CAZ, ceftazidime; CTX, cefotaxime; CFP, cefoperazone; FEP, cefepime; SAM, ampicillin–sulbactam; AZT, aztreonam; TZP, piperacillin–tazobactam; TIM, ticarcillin–clavulanate; IMP, imipenem; MER, meropenem; PB, polymyxin B; CT, colistin; DO, doxycycline; MH, minocycline; TGC, tigecycline.

[b] Statistically significant: *P* values ⩽0.05; ND: not determinable.

P1516 Impact of genetic surroundings on the function of integrons

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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 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-processive elements called mobilomes. This study focuses on a few integrons carrying aminoglycoside resistance and antifolate resistance genes and the variable stability of the cassettes integrated. The observations support that the level of integrase expression and the genetic surrounding both have strong influence on the operation of integrons. The aadA2 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 pLMO20. The formation of a composite element of IS6100 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.

P1517 Role of natural transformation in integron dissemination

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The rapid evolution of bacterial genomes associated with the widespread use of antibiotics has resulted in emergence and dissemination of antibiotic resistance genes, facilitated by horizontal transfer. Class 1 integrons are genetic units associated with multiple antibiotic resistance genes and have been associated with the ongoing spread of such genes. Identical integrons have been found in the chromosome of unrelated bacteria, suggesting the presence of mechanisms facilitating horizontal movement of integrons. Natural transformation is one of the mechanisms that allow 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 competent *Acinetobacter baylyi* BD413 was used as recipient bacterium and integron-carrying bacteria *Acinetobacter baumannii* 65FFC and 064, *Escherichia coli* 12FFC, *Morganella morganii* 235HUC, *Pseudomonas aeruginosa*, *Salmonella* rissen 486 and *Salmonella typhimurium* 490 were the donor used in natural transformation assays, performed by an in vitro protocol where a filter was used as a physical support. Transfer of class 1 integrons was screened by PCR with 5′CS and 3′CS primers. It was observed that integrons can be transferred in vitro by natural transformation at a low frequency, especially when present in plasmids. Presence of plasmids in donor bacteria and absence in transformants where transfer of integrons occurred lead to the hypothesis that plasmids may have an important role in integron transformation, but, after transfer, plasmids might be incorporated in the chromosome of the transformant or the integron may jump from the plasmid into the chromosome and the plasmid is lost by the cell. Natural transformation assays with transformants that previously acquired integrons as donor bacteria were also performed and high transformation frequencies were obtained. Sequencing of integron flanking regions shown that all integrons from both donor and transformant bacteria seem to be present on transposons. These experiments allowed concluding that, though natural transformation of integrons is rare, once a recipient bacterium has integrated an integron, it can be quickly disseminated, which could lead to its broad accumulation in the environment. Integrons that were transferred by natural transformation are incorporated in a stable way, even when the transformant is in an antibiotic-free environment.

P1518 Charecterisation of a complete DNA region surrounding non-mosaic tet(32) gene. Evolutive relation with the element carrying tet(W) gene

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Objective: A recent work about the description of DNA regions flanking the tet(32) gene suggested a high similarity with TnB1230 element carrying the tet(W) gene (Warburton, 2009). This result could have interesting implications because tet(W) has emerged as a widespread determinant, but because also Tet(32) confers slight increased tetracycline MIC. We wanted to know the real relationship between both regions carrying these tet genes in the most extended known region harbouring tet(32), to suggest the evolutive relationship between them and the ecological implications.

Material and Methods: One clinical isolates of *Acidaminococcus intestini* RYCMR95 were found carrying tet(32) gene as part of an epidemiological study. The DNA regions surrounding was known and an analysis of comparative genome with Genebank sequences allowed to define the element carrying tet(32). When the DNA region was define in Acidaminococcus, then was compared with the known genetic element carrying tet(W).

Results: A total of 15Kb were sequenced in RYCMR95 strain. However only 11.2Kb was identical in other species: *Clostridium scindens* and *Anaerotruncus colihominis*. The homology was 99% throughout 11.2Kb among them including the distance between the different orfs, suggesting a recent event of horizontal transfer. The homology of the flanking sequences to this region was below 50%. This 11.2Kb-region was characterised by the presence of a site specific recombinase, at 7Kb and a relaxase at 3.3Kb downstream of tet(32) respectively. An orf located at 362 bp upstream of tet gene showed homology to aminonucleoside antibiotic resistance. When this 11.2Kb-region was compared to TnB1230 of *Butyrivibrio* and ATE-3 of *Arcanobacterium pyogenes* which carry tet(W) gene, the homology was 78% between tet genes, 86% in 853 bp upstream including the orf with homology to aminonucleoside antibiotic resistance and 96% in 390 bp downstream. In other *A. intestini* and *A. fermentans* strains the presence of tet(32) and tet(W) was also detected.

Conclusion: These results suggest that the hypothetical element carrying tet(32) is the 11.2Kb and has a common ancestral element with elements carrying tet(W), although subsequently they diverged. This work suggest that the recombination between modules belonging to tet(W) element or

tet(32), could be easy and might contribute to the constant diversification observed in elements carrying tet(W) and probably similar a process could be described in element carrying tet(32).

Soft tissue, bone and joint infections

P1519 Skin and soft-tissue infection in patients with solid tumours

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Objective: 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 reccurrence, 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, has 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%), urinary tract cancer in 4 (6%), 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 (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 21.921, 95% CI: 2.970–161.815; $p = 0.002$) 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.

P1520 Factors affecting the duration of intravenous antibiotic therapy for cellulitis in an outpatient setting

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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 ($\leqslant$3 days) or extended course ($\geqslant$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 $\leqslant$3 days therapy, 32 had 4–6 days and 38 had $\geqslant$7 days. On univariate analysis (table 1) extended course treatment was associated with higher baseline CRP, ESR, creatinine, longer duration of cellulitis pre outpatient IV antibiotics, lower haemoglobin, increasing age, presence of diabetes and male sex. In the multivariate model, male sex ($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.

Table 1. Univariate factors affecting duration of treatment

	$\leqslant$3 day, n = 28 (%)	$\geqslant$7 days, n = 38 (%)	P value
Diabetes	0 (0)	8 (21.1)	0.0169
Male gender	13 (46.4)	31 (81.6)	0.0038
CRP (mg/L)	31.2	84.6	0.002
ESR (mm/hr)	20.4	46.8	<0.001
Creatinine (mmol/dl)	77.2	89.6	0.0423
Albumin (g/L)	37.4	32.2	<0.001
Haemoglobin (g/dl)	14.3	13.5	0.0423
Duration of symptoms pre treatment (days)	5.0	9.3	0.014
Age (years)	48.4	62.1	0.0013

P1521 Clinical and microbiological analysis of skin and soft tissue infections over five-year periods

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Objective: The incidence of skin and soft tissue infections (SSTIs) is uprising and community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) emerged as a significant causative pathogen. Yield of culture by needle aspiration or tissue biopsy is known to be not productive in SSTIs. The objectives of this study are analysis of clinical features and microbiological distributions of SSTIs at a tertiary hospital in Korea and comparison of yield as each method collecting the specimen for microbiological identification.
Methods: We retrospectively reviewed medical records of 760 adult cases of community-acquired SSTIs from September 2003 to August 2008 of Korea University Guro Hospital. Demographic findings, clinical features and microbiological distributions were evaluated. We compared the positive culture rate of each method: blind needle aspiration, ultrasound (US)-guided needle aspiration and US-guided tissue gun biopsy.
Results: The annual number of cases and admitted cases due to SSTIs per total number of inpatients (case number/1000 persons) has been increased for recent 3 years: 117, 1.93 from September 2005 to August 2006, 140, 2.12 from September 2006 to August 2007 and 213, 2.58 from September 2007 to August 2008. More than half of patients (52%) did not have suspected precipitating factor and 76.1% of patients did not have underlying disease. Repetitive cases were 10.0% and they tend to occur at same site with previous infection (86.8%) at an interval of 6 months to 2 years (52.6%). Mean height, weight and BMI were higher and more patients had cirrhosis in repetitive infection group ($p < 0.05$). Cirrhosis was an independent risk factor for repetitive infection (OR 3.64, 95% CI 1.36–9.76). Most common pathogen was methicillin-susceptible *Staphylococcus aureus* (MSSA) and CA-MRSA was cultivated at 4 cases (2 in 2003–2004 and 2 in 2006–2007). US-guided aspiration showed higher positive culture rate than blind needle aspiration (73.9% vs. 48.3%, $p < 0.05$). US-guided gun biopsy was not productive (positive culture rate, 17.5%).
Conclusions: Considering microbiological distribution of SSTIs in Korea, penicillinase-stable penicillin or 1st generation cephalosporin would be enough to use as initial antibiotic. In the cases with fluctuating lesion, US-guided needle aspiration was superior to blind needle aspiration for the identification of pathogens. Considering that there are occult abscesses in many cases, US examination would be valuable.

P1522 Common pathogens isolated in diabetic foot infection in a rural hospital

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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 scarping 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/clavoulanic. 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.

P1523 Diabetic foot ulcers: a bacteriologic study of 193 patients

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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 37ºC 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 (60.1%), *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.

P1524 Quinolone monotherapy in diabetic foot infections: rate of failure and selection for resistant strains

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Background: Quinolones are different for capability to select resistant strains during therapy. The in vitro measure for this characteristic is defined mutant prevention concentration. In vivo there are few data about quinolone monotherapy and rate of failure and emergence of resistant strains. For this purpose we review the files of patients with diabetic foot infections (DFI) treated with quinolone monotherapy, at Pisa Hospital in Italy from January 2006 and December 2008.
Methods: We compare the microbiological results of all patients with DFI that were treated with quinolone monotherapy.
Results: In the period studied, 167 patients were treated with ciprofloxacin monotherapy, 373 patients with levofloxacin monotherapy and 169 patients with moxifloxacin monotherapy. From the microbiological point of view levofloxacin was able to eradicate all bacteria in 40% of patients, moxifloxacin 32% and ciprofloxacin 22%. Selection of resistant bacteria among Gram positive and Gram negative was higher for levofloxacin (56%) with respect to moxifloxacin (43%) and ciprofloxacin (40%) (p:0.03 for moxifloxacin and p:0.02 for ciprofloxacin). Selection of resistant Gram positive strains was higher for levofloxacin (59%) with respect to moxifloxacin (41%) (p:0.03). Selection of resistant Gram negative strains was higher for levofloxacin (53%) with respect to ciprofloxacin (39%) (p:0.01).
Levofloxacin select *S. aureus* quinolone-resistant more easily than moxifloxacin (p:0.05). *P. aeruginosa* quinolone-resistant was selected more easily by moxifloxacin with respect to ciprofloxacin (p:0.01)
Conclusions: Quinolone monotherapy efficacy in eradicating bacteria in DFI is scarse. Levofloxacin is the most effective molecule but in case of persistance of bacteria in DFI, levofloxacin has the higher capability to select strains resistant to quinolone. In DFI, monotherapy with quinolone has to be reconsidered in the light of selection of resistant strains.

P1525 Clinical, biological and radiological differences between *P. aeruginosa* and other Gram-negative bacilli vertebral osteomyelitis

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Objectives: To describe clinical, biological and radiological findings as well as the prognosis of *P. aeruginosa* vertebral osteomyelitis (PAVO) in comparison with vertebral osteomyelitis produced by other Gram Negative Bacilli (OGNBVO).
Methods: Retrospective and descriptive study including 267 patients older than 14 years old diagnosed with pyogenic vertebral osteomyelitis (PVO) between January 1983 and November 2008 in two tertiary hospitals. Data were collected prospectively according to a specifically designed protocol. The diagnosis of VO was established by the presence

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, 72.7.1±33.19 days versus 42.8+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 BGNVO. In patients with VO, previous spinal surgery and skin or soft tissue infection should make considering *P. aeruginosa* as as a possible aetiologic agent. With an appropriate treatment, the prognosis of PAVO does not differ of others VO caused by GNB.

P1526 Epidemiological trends of empirical or microbiologically documented treatment of chronic osteomyelitis. Results from a ten-year survey at a reference bone and joint infectious diseases department

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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 6/2, 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 (⩽3months) 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.

P1527 Vertebral osteomyelitis by *Streptococcus* spp.

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Objectives: To study the epidemiologic, clinical and diagnostic features, biologic findings and outcome of all cases of vertebral osteomyelitis (VO) caused by *Streptococcus* spp.
Methods: Multicentre, retrospective/prospective, transverse and descriptive study of 514 patients diagnosed of VO. Inclusion criteria: 1) spinal inflammatory pain or fever and spinal pain on physical examination, 2) imaging techniques showing findings compatible with VO, 3) aetiologic diagnosis. We analyse the epidemiology factors, clinical and radiologic features, aetiological agents, and treatment and outcome. We followed all patients since 12 months.
Results: Pyogenic vertebral osteomielytis was diagnosed in 267 patients (52%) since January 1983 to November 2008. Of them, 17 cases (6.4%) were caused by *Streptococcus* spp. The median age of the patients was 65 years±11 IQR (10 cases >65 years old). Thirteen patients (77%) were male. Predisposing factors: (1) previous infection: 9 patients (53%); (2) immunosupresion status: 4 cases (23.5%); (3) previous bacteraemia: 4 cases (23.5%); (4) previous vertebral surgery: 3 patients (18%); and (5) diabetes mellitus: 2 patients (12%). Duration symptoms previous diagnosis: 30 days (15–180 range). Vertebral level affected: 8 lumbar (47%), 4 lumbosacral (24%), 3 thoracic (18%) and 2 cervical. Clinical features: spinal pain on physical examination 17 patients (100%), inflammatory pain 15 cases (88%), neurologic symptoms 11 patients (65%), fever 8 (47%) chills/rigors 7 (41%), and constitutional symptoms 6 (36%). Blood cultures were positives in 6/10 cases (60%), bone biopsy was positive in 10/11 (91%), and adjacent infectious foci cultures in 9/9 (100%). Causal agents isolated: *Streptococcus* spp. viridians group 7 (41%), *S. agalactiae* 4 (24%), *S. pneumoniae* 3 (18%), and others *Streptococcus* spp. 3 (18%). Paravertebral masses were detected in 10 cases (59%), epidural masses in 7 (41%), and psoas abscesses 2 (12%). Nine patients (53%) required surgical treatment. Of the 17 cases, 14 cases (82%) were cured, although 3 patients (18%) had severe functional sequels.
Conclusions: 1) VO caused by *Streptococcus* spp. occurred more frequent in elderly patients. 2) Previous infections at other site is frequent. 3) Diagnostic yield of microbiological techniques is very high. 4) Paravertebral and/or epidural masses are frequent. 5) Surgical treatment is frequently necessary. 6) Severe function al sequels are infrequent.

P1528 Osteoarthricular involvement in brucellosis: study of 155 cases

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Objectives: Despite the high morbidity rates of brucellosis in Turkey, its mortality rate is relatively low. Osteoarthiculary complications has been reported in 10–80% of all brucellosis cases. The aim of this study was to determine the rates, types and clinical features osteoarthricular involvement of 155 brucellosis cases.
Methods: 155 brucellosis cases admitted to our hospital between June 2003 and November 2008 were evaluated in terms of clinical and laboratory findings. The diagnosis was established on the basis of standard tube agglutination titre of 1/160 of antibodies for brucellosis

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 imagine in for patients who had lumbago 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 sacroiliitis and spondylodiskitis, one had sacroiliitis and peripheral arthritis, and the remainder sacroiliitis and bursitis. Spinal epidural abscess was found seven cases. Sacroiliitis 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 sacroiliitis and spondylodiskitis especially where this infection is endemic countries such as Turkey.

P1529 Clinical presentation and outcome of septic arthritis diagnosed in a community hospital

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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 frecuent 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 *Streptococcus* sp. (8%) and Gram-negative bacilli (7%). 8.3% of the cultures of synovial fluid were Polimicrobial. Bacteraemia was documented in 10% of patients but blood cultures were draw 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 morbi-mortality.
Conclusions: 1. Half of our patients had underlaying 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.
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P1530 Septic arthritis caused by anaerobic bacteria

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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 1733 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 traumatism 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 arthrotomy 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 anthritis 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

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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-Lemoshow $p = 0.18$).
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.

Significant independent variables associated with lower extremity amputation

Variable	Multivariable Odds ratio (95% CI)	*p* value
Male gender	1.37 (1.12, 1.68)	0.0024
Transfer from another acute care facility	3.44 (1.45, 8.19)	0.0053
Ulcer and "other" infection vs cellulitis	1.60 (1.26, 2.02)	0.0001
Surgical site infection vs cellulitis	3.32 (2.17, 5.10)	<0.0001
Albumin <2.8 g/dL	1.76 (1.34, 2.32)	<0.0001
PT/INR >1.2 or PT >14 seconds	1.89 (1.41, 2.52)	<0.0001
White blood cell count ⩾11,000/mm^3	2.72 (2.22, 3.32)	<0.0001
History of peripheral vascular disease	2.22 (1.80, 2.73)	<0.0001
History of previous lower extremity amputation	1.40 (1.13, 1.73)	0.002
Streptococcus monomicrobial infection	1.82 (1.20, 2.75)	0.005
Enterococcus monomicrobial infection	2.56 (1.38, 4.75)	0.0028
Gram– (non-*Pseudomonas*) monomicrobial infection	2.60 (1.55, 4.34)	0.0003
Any type of polymicrobial infection	1.74 (1.38, 2.19)	<0.0001

Epidemiology and clinical aspects of infections

P1532 *Actinobaculum* spp.: clinical observation of 19 cases

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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 (bioMerieux) 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 an average of 2.7 days (range 0–13 days), due to the diminished sensitivity of *Actinobaculum* spp. to the commonly used antibiotics in UTI (ciprofloxacine and sulfamethoxazol/thrimetoprime) and to the length of microbiological diagnosis. 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.

P1533 Community-acquired *Stenotrophomonas maltophilia* infections: a systematic review

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Objective: *Stenotrophomonas maltophilia* is a pathogen that causes infections mainly in immunocompromised patients. However, community-acquired *S. maltophilia* infections have been occasionally reported.
Objective: 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 77 patients with community-acquired *S. maltophilia* infections 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.

P1534 Seasonal patterns of invasive *Streptococcus pyogenes* disease in the Northern hemisphere

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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 Canada (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 autumnal 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.

Distinct 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

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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 (INCDMIC), 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 prepared 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 requiering tracheostomy.
Conclusions: In Romania, type B botulinic 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

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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%), Streptococci 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 suggest 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

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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 testes included Ampicillin (AM), Co-trimoxazole (SXT), Ciprofloxacin (CI), Tetracycline (TC), Erythromycin (EM), Choleramphenicol (C), cefexime (CFM) 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 21 cases were Ogawa. Disease reported from thirteen provinces. The majority of cases were from Tehran, Qum and Zahedan with 56, 26 and 25 cases respectively. 24 (11%) of patients were under 15 years old and 196 (89%) of patients were older than 15 years. 149 (68%) 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 chloramphenicol. All isolates were susceptible to tetracycline, ciprofloxacin, and erythromycin. MIC ranged1–1.5 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, nalidix acid and furazolidon.

P1538 Peritonitis in continuous ambulatory peritoneal dialysis: five-year experience in our hospital

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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 was 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 (bioMercieux, 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 effluent in 104 (100%) cases, abdominal pain in 92 (88.4%), rebound tenderness in 87 (83.6%), fever in 74 (71.2%) and nausea/vomiting in 56 (53.8%) cases. Peripheral 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 positivity of peritoneal fluid culture was 77.9%. The microorganisms isolated were: coagulase-negative staphylococcus in 51 (62.9%) cases (*S. epidermidis* in 84.3% of cases and *S. hominis* and *S. haemolyticus* in the rest of cases), *Staphylococcus aureus* in 7 (8.6%), Escheriscia Coli in 6 (7.4%), *Klebsiella* spp. in 5 (6.2%), *Pseudomonas* spp. in 5 (6.2%), *Streptococcus* spp in 3 (3.7%), *Enterobacter* spp, in 2 (2.5%) and *Candida albicans* in 2 (2.5%) cases. All patients took empirically antibiotic therapy. In 36 (34.6%) cases the initial therapy was changed according to antimicrobial susceptibility. 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 causes in our patients. Systematically registration of migroorganisms related with peritonitis of CAPD and their antimicrobial susceptibilities will be helpful for earlier and targeting treatment.

P1539 Epidemiological changes in the human brucellosis

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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 189 *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+ 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 antisera. 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.

P1540 Are measles still a problem? Istanbul, Turkey data

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Measles is an infectious disease documented since the early years of 20th century in Turkey. Measles was included to required to be reported diseases list in 1930 and nine months old infants were began to get 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.

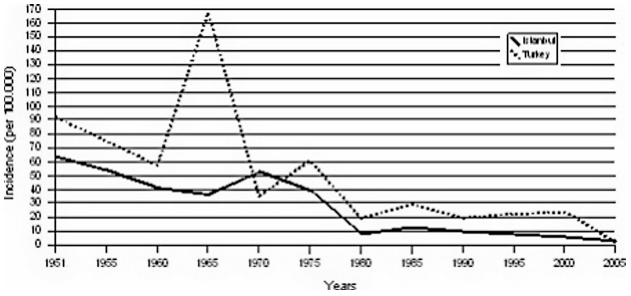
Comparison of annual measles incidences between 1951–2005

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 campaigne 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 sourced case.

Measles elimination in Istanbul can be declared as already provided as a consequence of determined and effective realisation of measles vaccination campaigne which was planned to get by 2010 at first.



P1541 Epidemiological and clinical features regarding *Rickettsia conorii* infection in Romania

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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 Bals". 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 Constanta (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 endemicity 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.

P1542 Lymphadenopathy due to uncommon infectious agents: *Coxiella burnetii*, *Rickettsia typhi/conorii*, and *Bartonella henselae/quintana*

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Objectives: Although the finding of lymphadenopathy sometimes raises fears about serious illness such as lymphoma, acquired immunodeficiency syndrome, metastatic cancer, or mononucleosis syndrome it is, usually a result of benign infectious causes. The aim of this study was to investigate whether there is an association between lymphadenopathy and infection with *Coxiella burnetii*, *Rickettsia conorii/typhi*, and *Bartonella henselae/quintana*.
Methods: 280 patients who were diagnosed with lymphadenopathy (the typical causes were excuded by clinical evaluation and confirmatory laboratory tests) in AHEPA University Hospital, from 2004 to 2008, were prospectively studied. Characteristics of the patients were collected including age, sex, underlying disease, occupation, contact with animals, consumption of unpasteurised products and the histopathological results in the cases where a lymph node biopsy had been performed.
Paired serum specimens were obtained from all patients and IgG/IgM antibody titers against *Coxiella burnetii*, *Rickettsia conorii/typhi* and *Bartonella henselae/quintana* were assessed by immunofluorescence assay (Focus, kit). A serological diagnosis was made on the basis of either evaluated titers of IgM antibodies >1:20 or IgG antibodies >1:256, or the presence of a fourfold rise in the serum IgG titer between acute and convalescent phase.
Results: The patients tested negative for the presence of *Rickettsia conorii* or *typhi* infection. In 70 patients (29 adults/41children) the diagnosis of *Bartonella henselae* infection was confirmed. In 15 out of 29 adults and in 18 out of 41 children contact with cats was reported. In two cases a contact with dogs was also reported. In five patients the histological examination of lymph nodes pointed out histopathological findings compatible with cat scratch disease. Acute Q fever was identified in four cases. Two patients had a history of recent exposure to possible sources of *C. burnettii*.
Conclusions: *Bartonella henselae* infection is a common cause of lymphadenopathy. A definitive diagnosis should be made by laboratory examination, while history and physical examination and clinical evaluation may strongly suggest a particular cause.

P1543 Differences in clinical features between the Boryoung and Karp genotypes of *Orientia tsutsugamushi*

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Background: Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi* (*O. tsutsugamushi*) and transmitted through the bites of thrombiculid mites. The aim of this study was to investigate whether there are any differences in clinical features and severity between the Boryoung and Karp genotypes.
Methods: Nested polymerase chain reactions (PCR) were performed with the blood buffy coats or eschars of patients with suspected scrub typhus who visited Chosun University Hospital from September to December in both 2005 and 2006. We compared the clinical features and severity of the patients who were confirmed by nested PCR to have the Boryoung and Karp genotypes.
Results: Of 145 patients definitively diagnosed with scrub typhus, 132 were positive by nested PCR. Of these 132 patients, 120 were clustered as having the Boryoung genotype and nine as having the Karp genotype. Eschars were observed in 97.5% of the patients with the Boryoung genotype and in 66.7% of patients with the Karp genotype, and the difference was statistically significant. However, there were no significant differences in complication rate, need for intensive care or mean length of hospital stay.
Conclusions: We found that 97.5% of patients with the Boryoung genotype had eschars, compared with only 66.7% of those with the Karp genotype. To the best of our knowledge, this is the first report of suggestion that rate of eschar formation may depend on genotype.

Molecular biology – part 2

P1544 Molecular epidemiology of human papillomavirus in Madrid, Spain

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Objectives: Human papillomavirus (HPV) are the most common sexually transmitted infections worldwide. Persistence of high-risk HPV genotypes is a significant risk factor for the development of cervical cancer. The main goal of the present study was to investigate the prevalence and genotype distribution of HPV in Madrid (Spain) during 2006–2008.
Methods: We present a retrospective study including samples obtained from 7 different hospitals in Madrid in the last three years involving women with an abnormal cervical cytology. To detect the HPV in the different cervical cell specimens, we have used the Linear Array HPV Genotyping Test (Roche Diagnostic). The test utilises amplification of target DNA by PCR and nucleic acid hybridisation detecting up to thirty seven HPV DNA genotypes including high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and low-risk genotypes (6, 11, 40, 42, 44, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 84, IS39, CP6108).
Results: In this study we have analysed a total number of 1551 samples. The overall HPV prevalence was 77% from cervical samples collected. HPV genotypes distribution was widely heterogeneous with up to 37 different genotypes identified. High-risk and low-risk genotypes were found in 53% and 47% of all cases, respectively. Among these HPV types, genotype 16 was detected in 14% of the patients whereas genotype 18 was only detected in 3% of the patients. Our data showed that up to 68% of the HPV high risk genotypes detected, belonged to genotypes not included in the HPV vaccine. Co-infection with multiple HPV types was detected in 38% of the women included in our study. The presence

of two, three and four different HPV genotypes infecting the same individual was detected in 56%, 25% and 12% of the co-infection cases respectively. Furthermore, more than five different genotypes were found in 8% of the cases.
Conclusions: According to this study, HPV prevalence was markedly high in women presenting cervical cellular alterations. HPV genotypes 16 and 18 were detected in less than 20% of the patients although we have found a large percentage of cases belonging to genotypes not covered by the quadrivalent vaccine. Overall, co-infection with multiple HPV types was a common finding.

P1545 Performance of the Gen-Probe APTIMA COMBO 2® assay on the Panther analyser

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Objectives: The objective was to evaluate the analytical performance of the Panther; a new 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 $\leqslant$10% at 5 fg/reaction and $\leqslant$15% at 0.5 fg/reaction. Within-run precision was $\leqslant$5% for NG at 250 and 50 fg/reaction. The within-run precision for the dual-positive panel member was $\leqslant$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.

P1546 An automated magnetic extraction system for *Chlamydia trachomatis* DNA, tested with the Roche COBAS® TaqMan detection system

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Objective: *Chlamydia trachomatis* accounts for more cases of sexually transmitted infection than any other bacterial pathogen. Methods of detection include culture or antigen detection, both now superseded by amplification reactions. This method is advantageous offering superior sensitivity and specificity and is much quicker to perform. The aim of the study was to evaluate the performance of the automated NorDiag BUGS'N BEADS™ STI-fast kit on the NorDiag Bullet instrument using swabs collected and transported in M4RT® MicroTest Culture Transport System (Remel, Inc) media with the aim to CE IVD the process. The study was carried out at Dumfries and Galloway Royal Infirmary which serves a population of 146,000. In 2007–08, the total number of *Chlamydia* tests performed in the region was 7202 with a prevalence rate of 8.8%.
Methods: 704 swabs were tested in parallel using two different extraction methods detailed below. The performance of the BUGS'N BEADS™ STI-fast kit on the Bullet system was compared to the nucleic acid extraction using the Roche AMPLICOR® CT/NG specimen preparation kit on the Tecan Freedom EVO75 instrument. The BUGS'N BEADS™ STI-fast method is based on nucleic acid extraction using magnetic beads that bind to bacteria and are then magnetically removed from the sample matrix allowing for concentration of the sample, as lysis occurs on the captured cells only. This differs from the Roche specimen preparation kit as it uses detergents to release Chlamydial DNA from *Chlamydia* reticulate bodies in preparation for amplification.
Detection of Chlamydial DNA for both extraction methods was performed with the COBAS® TaqMan CT test v 2.0 on a COBAS® TaqMan 48 analyser. Samples with discrepant results from the two methods were reprocessed in duplicate for both methods. A true positive result was then based on two out of three results.
Results: A total of 704 specimens were tested in parallel. The overall prevalence rate based on true disease status was 5.7%. Table 1 shows the overall results of the study including inhibition.
Conclusion: The results of this study demonstrate that the BUGS'N BEADS™ STI-fast kit exhibits excellent sensitivity and specificity values comparable with the Roche system. Inhibition is significantly reduced; therefore decreasing the number of repeat tests resulting in quicker turn around times. Another benefit of the NorDiag system is the automated sample barcode reader which reduces the possibility of transcription error.

Table 1. Overall results

Results	True status			
M4RT swab samples	Roche COBAS® TaqMan® CT Test, v2.0 manual method		BUGS'n BEADS™ STI-*fast* on NorDiag Bullet	
	+	−	+	−
Test +	39	3 (F+)	40	3 (F+)
Test −	2 (F−)	660	1 (F−)	660
Sensitivity	95%		97.5%	
Specificity	99.5%		99.5%	
PPV	92.8%		93%	
NPV	99.6%		99.8%	
Inhibition	39		10	

P1547 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 nomenclature 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 matrixes (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%. Thirteen different methods were used with a percentage of correct results ranging from 14 to 100%. For swabs, 97 labs participated with a percentage of good results of 89% with no false positive results and 13% of false negative results. The percentage of clinically relevant errors was 1.03%. For *N. gonorrhoeae*, 24 labs participated. The percentage of good results was 78.75%. The sensitivity and the specificity were 80.7%and 71%, respectively. The percentage of false positive results was 24%. Three types of negative samples were used: a blank sample, a sample containing *N. cinerea* and a sample containing *N. lactamica*. All false positive results came from the two latter samples.
Conclusion: For the detection of *C. trachomatis* in urine, the survey revealed a problem of sensitivity. For *N. gonorrhoeae*, a problem of specificity occurred especially with some commercial kits. Therefore, this kind a study allowed thus 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

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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 able to detect seven viral and four bacterial pathogenic species simultaneously. In this report we evaluate the MLPA as a diagnostic 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 *Chlamydophila 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 titers 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 for a diagnostic laboratory that combines a short turn-around time and cost effectiveness with a good sensitivity and specificity.

P1549 Lower respiratory viral infections in hospitalised adults

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Objective: Lower respiratory tract infections (LRTIs) are a leading cause of hospitalisation, morbidity and mortality, particularly in patients with immunosupression and/or underlying conditions. The aim of this study was to evaluate the usefulness of molecular diagnostic methods and to assess the prevalence of respiratory viruses (RVs) in hospitalised adults who underwent a bronchoalveolar lavage (BAL) for an acute respiratory event.
Methods: Over a 1 year period (2007–2008), 118 BAL samples were prospectively collected from 95 patients (35 females; mean age: 57) with community- (n: 72) or hospital-acquired (n: 46) severe pneumonia. Viral isolation was routinely performed by conventional culture (MRC-5 cell line) and 48 h shell vial cultures (Vero, A549, HEp-2 and MDCK cell lines). Molecular methods included two in house multiplex RT-PCR nested assays for simultaneous detection of 14 RVs and a single in house RT-PCR nested assay for human metapneumovirus (hMPV) identification. Conventional microbiological tests for bacteria, micobacteria, fungi and parasite detection were routinely performed.
Results: Twenty three RVs were identified by molecular methods in 22 of 118 BAL specimens (18.6%). Rhinovirus was the most frequently detected virus (n:11, 47.8%) followed by influenza virus A (n:2) and hMPV (n:2). Respiratory syncytial virus (RSV) A, RSV B, parainfluenza virus (PIV) 1, PIV 2, PIV 3, PIV 4, adenovirus (ADV) and enterovirus were each identified in one case. In addition, 11 cytomegalovirus and 5 herpes simplex virus type 1 (HSV 1) were isolated by viral culture. Except for ADV, no other RV could be detected by culture. Mixed infections were found in 13 of 22 (59.1%) RVs positive BAL specimens. In other 2 cases viral co-infection was detected (PIV 3 + rhinovirus, PIV 1 + HSV 1). RVs were mainly identified in patients with community-acquired LRTIs (18/72, 25.0%). An immunosuppressive and/or underlying condition was present in all except one of the patients with viral infection.
Conclusions: RVs were frequently detected in BAL specimens from hospitalised adults, particularly in those with LRTI of community acquisition. Although these viruses were mainly found in mixed infections, its presence in the lower respiratory tract suggests a pathogenic role. Molecular assays enabled multiple, sensitive and rapid identification of RVs and should be included as routine diagnostic procedures.

P1550 Application of an expanded gold standard and latent class analysis to decide on the strategy for the diagnosis of *M. pneumoniae* infections

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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 EIAs 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%. The specificities ranged from 80–99% for IgM and from 84–89% for IgA both in the acute phase and in the 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

by LCA. Sensitivities and specificities of PCR and NASBA alone were 79% and 76%, and 98% and 97%, respectively. In combination with a second or a third test, sensitivity increases to 92% and 95%, respectively. Results of LCA confirmed the results obtained with the use of an EGS.
Conclusion: Evaluation of the 4 EIAs and 2 independent amplification tests for MP showed substantial differences between the performances of the assays. Amplification methods are the most sensitive tests to diagnose an acute MP infection. IgM and IgA antibodies are of limited value. IgG serology has usually a moderate sensitivity but results in late diagnosis. The combination of different tests increases the specificity and sensitivity.

P1551 *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* EQA Pilot Study

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Objectives: To assess the proficiency of laboratories in the correct detection of *C. pneumoniae* and *M. pneumoniae* by nucleic acid amplification techniques (NAATs).
Methods: This EQA panel consisted of a total of 13 samples in BAL or transport medium: 6 samples containing various concentrations (4.9–490 IFU/ml) of *C. pneumoniae* (ATCC VR-1355), 5 samples containing various concentrations (20–5000 CCU/ml) of *M. pneumoniae* (ATCC 29085) and 2 samples negative for both.
Results: 79 laboratories from 18 countries participated in this EQA study. 64 datasets were returned for *C. pneumoniae* (n = 5 conventional commercial, n= 10 conventional in-house, n = 4 real-time commercial, n= 43 real-time in-house, n = 2 SDA). 67 datasets were obtained for *M. pneumoniae* (n = 5 conventional commercial, n= 10 conventional in-house, n = 4 real-time commercial, n= 46 real-time in-house, n = 2 SDA). For the total panels, correct results per sample varied between 95.3% and 100% for *C. pneumoniae* and between 53.7% and 98.5% for *M. pneumoniae*. Correct results for positive and strong positive samples for both *C. pneumoniae* and *M. pneumoniae* ranged between 88.1% and 100%. 95.3% and 98.4% correct results were obtained for samples with the lowest *C. pneumoniae* input (4.9 IFU/ml). 53.7% and 55.2% correct results were obtained for samples with the lowest *M. pneumoniae* input (50 CCU/ml). In general, commercial conventional NAATs showed sensitivity problems compared to conventional in-house NAATs for both organisms. On the other hand, real-time commercial NAATs scored better than real-time in-house assays in terms of sensitivity for both organisms. For *C. pneumoniae* and *M. pneumoniae*, 2.0% and 2.4% false-positive results, respectively, were reported.
Conclusion: Analysis of the data for *C. pneumoniae* showed that the concentrations used were easily detectable by the vast majority of participants with percentages of correct results ranging from 95.3–100%. The percentage of correct qualitative results for *M. pneumoniae* demonstrated that the range of concentrations included in this panel proved challenging for a number of participants. False negative results were the most important problem for *M. pneumoniae* positive samples. The inclusion of low positive *M. pneumoniae* samples allows also to evaluate the sensitivities of individual tests.

P1552 Evaluation of three commercial serological tests versus real-time PCR for diagnosis of *Mycoplasma pneumoniae* infection in children

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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 culture is time consuming, can be influenced by previously administered empiric 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 (Pneumoslide IgG-IgM, Vircell, Granada, Spain) and 4) by a rapid ELISA-IgM (Immunocard *Mycoplasma*, Meridian Bioscience Inc., Cincinnati, Ohio, USA).
Results: MP was detected in 30 children out of 102 (29.41%) 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 IFT 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.

P1553 Evaluation of a real-time PCR assay for the molecular identification of *Pneumocystis jirovecii* pneumonia

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Objectives: *Pneumocystis jirovecii* an opportunistic fungal pathogen, causes pneumonia in HIV and immunocompromised patients. We evaluated the performance of a commercial real-time PCR assay FXG®: RESP (Asp+), (Myconostica) by comparing it with two routine microscopic tests for the detection of *P. jirovecii* from respiratory specimens.
Methods: 363 respiratory specimens, 78% bronchial washing (BW) or bronchoalveolar lavage (BAL) specimens, 17.6% sputum (SP), and 4.4% other respiratory samples. The microscopic tests were calcofluor white(FF) (Fungi-Fluor®, Polysciences, Inc) and immunofluoroscence (IFA) (MonofluoTM, Bio-Rad). DNA was extracted using the MycXtra® Fungal DNA Extraction Kit (Myconostica). PCR was set up using the FXG®: RESP (Asp+) Kit (Myconostica). Amplification, detection, and interpretation were performed using the AB7500 Real-time PCR system. Samples were considered positive if the *P. jirovecii* Ct value was $\leqslant$38.5 and negative if *P. jirovecii* Ct value was >38.5 with a valid IAC value.
Results: The positivity rate of PCR, FF and IFA was 15.7%, 7.2% and 8.3%, respectively. 80.7% of PCR positive results were from BW and BAL, 14% were from SP specimens. Compared to either FF or IFA, the PCR assay showed 91.9% sensitivity, 92.9% specificity, 59.6% PPV and 99.0% NPV. 3 samples were negative by PCR but positive by microscopic tests, 23 samples were positive by PCR but negative by microscopic tests. As all the specimens were from patients who had a clinical suspicion of pneumocystis pneumonia as a prerequisite for testing, we consider that these 23 samples most likely reflect increased detection of *P. jirovecii* by a more sensitive assay, rather than false positives. Therefore, in our patient population, the PCR assay is 118% more sensitive than FF and 89% more sensitive than IFA. When comparing test performance based on the type of specimens we found the test performance was better using BAL and BW specimens (93.8% sensitivity, 93.6% specificity, 65.2% PPV and 99.1% NPV) than using SP specimens (66.7% sensitivity, 90.1% specificity, 25% PPV and 98.2% NPV).
Conclusions: Our findings demonstrated that the FXG®: RESP (Asp+) assay was more sensitive than standard microscopy using calcofluor white (FF) or IFA for the detection of *P. jirovecii* from patients with possible pneumocystis pneumonia. Although, the assay can be applied to a variety of respiratory specimens, BW and BAL appear to be the optimal specimens for this molecular testing.

P1554 Use of optical mapping to identify prophages involved in virulence in *Pseudomonas aeruginosa*

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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

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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 multidrugresistant (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.

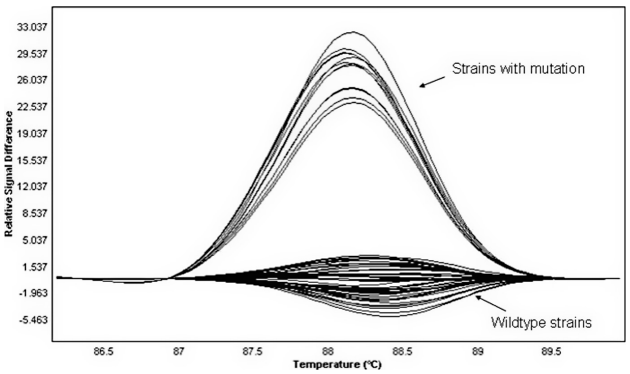

Figure: V176F mutation.

P1556 A prospective cross-over study demonstrating that rapid MRSA screening leads to a reduction in the rate of MRSA acquisition

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Objectives: To determine the impact of rapid MRSA screening on the transmission of MRSA whilst controlling for other variables.
Methods: A prospective, cluster, two period cross-over design on 7 surgical wards was used. For the first 8 months, 4 wards used rapid MRSA screening and three wards used a standard culture method. The groups were reversed for the second 8 months. Regardless of the method of detection all patients were screened on admission and then every 4 days. MRSA control measures remained constant.
Results: A total of 12,682/13,952 patient ward episodes were included in the study, with characteristics of the patient groups across the study periods being comparable. Admission screening identified 453 (3.6%) MRSA positive patient ward episodes, with a further 268 (2.2%) acquiring MRSA. A log-linear Poisson regression model was used to compare MRSA acquisition rates. Both length of stay on the ward and the proportion of emergency procedures carried out on a ward significantly increased the likelihood of MRSA acquisition. After adjusting for these and other variables, rapid screening was shown to reduce MRSA acquisition with patients being 1.49 (95% confidence interval 1.115–2.003; $p = 0.007$) times more likely to acquire MRSA in wards where they were screened using the slower culture method.
Conclusions: Screening of surgical patients using rapid testing resulted in a statistically significant reduction in MRSA acquisition. This result was achieved in a routine surgical service with high bed occupancy and low availability of isolation rooms, making it applicable to the majority of health-care systems worldwide.

P1557 The impact of rapid PCR screening for methicillin-resistant *Staphylococcus aureus* on patient isolation

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Objectives: This study aimed to determine the diagnostic accuracy of the GeneXpert Xpert MRSA™ real-time PCR assay (Cepheid) and whether

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 37ºC. 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.

P1558 Evaluation of commercially available molecular and culture-based assays for rapid detection of methicillin-resistant *Staphylococcus aureus*

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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 MSSA, 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 (10^5 CFU/ml for GeneOhm and GeneXpert, and 10^8 CFU/ml for Baclite according to manufacturer's instructions), while serial dilutions (10^1 to 10^3 or 10^4 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 (10^3 CFU/ml) concentrations (Table).

Table. Performance of three commercial assays for rapid detection of MRSA

Assay	Mean sensitivity (95% CI) at MRSA concentration		Mean specificity (95% CI)	Minimum limit of detection
	10^3 CFU/ml	10^4 CFU/ml		(CFU/ml)
Using isolates as samples				
GEneOhm™ MRSA (BD Diagnostics, BE)	93.7% (80.2–98.2)	ND*	77.2% (60.0–88.5)	140
GeneXpert™ MRSA (Cepheid, FR)	86.8% (75.2–93.5)	99.5% (97.9–99.9)	82.3% (55.2–94.6)	140
Baclite™ MRSA (3M, USA)	40.1% (26.1–55.9)	93.8% (86.7–97.2)	98.2% (90.5–99.7)	410
Using mixtures as samples				
GEneOhm™ MRSA (BD Diagnostics, BE)	97.6% (90.6–99.4)	ND**	55.6% (31.9–77.1)	44
GeneXpert™ MRSA (Cepheid, FR)	94.8% (88.3–97.8)	99.8% (99.2–100)	63.2% (38.7–82.4)	27
Baclite™ MRSA (3M, USA)	26.9% (14.8–43.8)	89.3% (77.7–95.2)	95.3% (81.7–98.9)	2400

*ND, Not determined. Only samples negative at the lower concentration (n = 2) were tested at this concentration.
**Not determined as all MRSA-positive mixtures were detected at the lower concentration.

Of the 27 MRSA tested, 2 strains that could not be detected at 10^3 CFU/ml were positive at 10^4 CFU/ml, giving a 100% positivity for GeneOhm at the latter concentration. Mean sensitivity for GeneXpert was ⩾100% at 10^4 CFU/ml with narrow CIs indicating high precision of this parameter estimate. Baclite showed the highest mean specificities for both mixtures and isolates. False-positive results with the 2 molecular tests were primarily due to MRCoNS. Minimum LoD for both molecular assays was similar for isolates, while GeneXpert could detect upto 27 CFU/ml of MRSA in mixtures.
Conclusions: A general increase in mean sensitivities of all three assays was observed with increasing MRSA concentrations. GeneOhm and GeneXpert showed comparable performance in terms of sensitivity, specificity and LoD.

P1559 Clinical evaluation of four molecular methicillin-resistant *Staphylococcus aureus* tests: Becton Dickinson GeneOhm, Hain GenoType MRSA Direct and two in-house assays

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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 real time PCR 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 retested 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

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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 antibiotherapy 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%), antibiotherapy (28.8%), 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, aproximately. 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/or 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

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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, Bilthoven, 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 t011 and t108. These are animal farm related types. The other spa-types obtained from this region were isolated only once or twice.
Conclusions: 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.

P1562 Cost-efficacy of rapid diagnostic testing of methicillin-resistant *Staphylococcus aureus* with the GeneXpert® system

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Objectives: Pre-emptive isolation of high risk patients is a cornerstone of the Dutch MRSA control strategy. However, most high risk patients are not colonised and remain in isolation for 3 to 5 days awaiting conventional culture results. We determined cost-efficacy of adding rapid diagnostic testing (using the Xpert MRSA assay on the GeneXpert® system) to the current MRSA control policy in a prospective multi-centre study.
Methods: All patients with increased risk of MRSA colonisation and fulfilling the criteria for pre-emptive isolation in 9 participating hospitals between 04/07 and 06/08 were eligible. In addition to the standard set of conventional microbiological cultures, MRSA PCR was performed directly on patient material. Infection prevention measures were based upon immediate PCR results, and, were thus withdrawn when negative. Culture results were used as reference.
Results: 917 patients (mean 48 years and 56.9% male) were enrolled, mainly because of previous hospitalisation abroad (64.4%), contact screening related to MRSA positive index patients (18.5%) and professional contact with pigs (14.7%). 2989 PCRs were performed (3.3 per patient including nose, throat, perineum), of which 158 (5.3%) yielded unresolved results. MRSA prevalence (based upon culture results) was 3.7%. Compared to conventional culture results sensitivity, specificity, positive predictive value and negative predictive value of the MRSA PCR were 76.5%, 94.6%, 35.1% and 99.1% respectively. In 779 (85.0%) patients pre-emptive isolation was discontinued upon negative PCR results. Median turn around times (TOT) between start of pre-emptive isolation and notification of PCR and culture results were 13.7 hours (range between hospitals 6.3 to 27.0 hours) and 87.6 hours (range 74.0 to 131.7 hours), respectively. Median TOT between start of isolation and discontinuation of isolation was 15.4 hours. Costs of the PCR were €67 per test (on average €221.10 per patient). We performed an economic evaluation (abstract number 718) and estimated the costs of an isolation day to be €27.30. Therefore adding MRSA PCR to the current policy will not be cost saving.
Conclusion: In a low endemicity setting, guiding of pre-emptive isolation upon the Xpert MRSA assay on the GeneXpert® system is safe and reduces pre-emptive isolation time to 15.4 hours at the costs of €221.10 per patient.

P1563 Direct detection of methicillin-resistant *Staphylococcus aureus* in broth-enriched clinical samples using a real-time multiplex PCR

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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 detect 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 Miniprep 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 12–14% of samples analysed with nuc screening PCR. The automated DNA preparation on the Tecan Miniprep 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.

P1564 Multiple-locus variable-number tandem repeat analysis in a setting of polyclonal endemicity of methicillin-resistant *Staphylococcus aureus*

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Objectives: The mehicillin-resistant *Staphylococcus aureus* (MRSA) population in the Hospital Universitario Nuestra Señora de Candelaria (HUNSC) 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 HUNSC.
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, sspA, 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 MLVA 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 HUNSC.

P1565 Comparisons of healthcare-associated and community-associated MRSA strains using SCCmec EVIGENE and routine susceptibility testing

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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 strains 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 (conferring 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 (pls) 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,

clindamycin, erythromycin and gentamicin than CA-MRSA, especially PVL-positive CA-MRSA.

P1566 Molecular typing of enterotoxigenic strains of *Staphylococcus aureus* isolated from different foods of animal origin

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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 synthesize 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 genetically 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 SEs 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 SmaI 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 hygienic 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 ses genes and whether these strains are more likely to synthesize 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

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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

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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 h/VISA. Among MRSA 3 strains were VISA (MIC = 3 mg/L) and 10 isolates h/VISA. PAP confirmed heteroresistant subpopulations in the presence of 4 mg/L of vancomycin. By spa typing, MSSA showed high heterogeneity while among MRSA three main groups were identified: t041(23 isolates), t008(19 isolates) and t001(9 isolates). Out of 3 VISA, 2 were assigned to spa type 041; of 10 h/VISA, 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)5.
Conclusions: VISA and h-VISA were detected only among MRSA. The majority of VISA and h-VISA were assigned to spa type t041 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

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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 dermathopytes 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-SCCmecV-t011 and ST1-SCCmecII-t127, being both isolates negative for the Panton Valentine leukocidin. The ST398 strain showed resistance to penicillin, oxacillin, tetracycline, erythromycin, clindamycin and telithromycin 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, ermB, tetL, ant(4′)(4”), aph(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 nasal colonisation status of the 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-SCCmecV-t108), father (ST398-SCCmecV-t108), mother (ST398-SCCmecV-t108 and ST1-SCCmecII-t127), and brother (ST398-SCCmecV-t011). Most of ST398 nasal isolates presented the phenotype of resistance that included β-lactams, macrolides, clindamycin, telithromycin 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 y 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.

P1570 Epidemiology of methicillin-resistant *Staphylococcus aureus* in the dermatology department of a university hospital, Casablanca, Morocco

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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. These strains were characterised by testing susceptibility to 16 antibiotics using agar disk diffusion method according to CLSI guidelines. The methicillino 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, Pefloxacin, Trimethoprim-sulfamethoxazole, 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 antibiotherapy for MRSA infection treatment.

P1571 Present prevalence of Panton-Valentine leukocidine-positive methicillin-resistant and susceptible *Staphylococcus aureus* in Spain

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Objectives: At present, the prevalence of Panton-Valentine leukocidine-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 Madrid (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. PFGE 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 erythromycin 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.

P1572 Prevalence of genes encoding pyrogenic toxin superantigens, the Panton-Valentine leukocidin and exfoliative toxins in *Staphylococcus aureus* isolated from furunculosis – association with agr group

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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), exfoliative toxins (eta, etb, etd), pyrogenic toxin superantigens (SAg) comprising TSST-1 (tst) 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 genes (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, seu and most additionally seb), 28% – agr I (most carried seg, sei, sem, sen, seo, single seg, sei, sem, sen, additionally seb, sel, seh, sek, seq or none

SE gene), 14.7% – agr III (seg, sei, sem, sen, seo, seu or none, one strain: tst and sea, one: eta), 2.6% – agr II (one: sec, one: sej, ser, sep). 84% strains (all from agr IV and most from the other groups) possessed lukS-lukF genes. The one MRSA (mecA+) strain possessed agr I, seg, sei, sem, sen, seo, seu and seb, sek, seq.
Conclusion: The great variety of virulence genes among *S. aureus* isolated from furunculosis cases has been observed. More than half of isolates were classified as agr IV, only single to agr II. Most strains possessed lukS-lukF genes and gene cluster: seg, sei, sem, sen, seo, seu. Only single strains carried tst and eta genes.

P1573 Characterisation of Panton-Valentine leukocidine positive methicillin-resistant *Staphylococcus aureus* in Italy

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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: t008 (5), t44(1), t319(1), t755(1), t2453(1), t2526(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, 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 (atl).
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.

P1574 Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a northern Italy hospital

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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 specialistic surgical units, general and specialistic 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%), endovasal catheter (8%), wound infections (8%), bronchoalveolar lavage (5%), tracheal aspirate (4%), cerebrospinal fluid (3%), perithoneal fluid (2%), drainage (1%), biopsy (1%), bile (1%), pericardial fluid (1%), transplant organ preservation fluid (0.5%) and perithoneal exhaust (0.5%). All isolates were analysed by PFGE, rep-PCR, SCCmec typing, PVL-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 PVL (0.9%). Overall, 20 spa types were detected, with t242, t008 and t001 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 β-lactams resistance, the strain is resistant to ciprofloxacin, levofloxacina 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 t001 (SCCmec type I). Our hospital is the first in Italy, where t242 reached an endemic level.

P1575 Clonal study of methicillin-resistant *Staphylococcus aureus* isolates of nosocomial origin in a Spanish hospital

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Objectives: Analysis of susceptibility to a wide panel of antibiotics of MRSA strains obtained from hospitalised, infected patients. Restriction profile 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 SmaI and analyzed by PFGE. Macrorestriction 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 $\leqslant$6 bands, were used to define a cluster.
Results: All strains presented MIC90 $>$ 512 to oxacillin, cefoxitin, cefotaxime, ceftazidime, cefepime, erytromicyn and clindamycyn. MIC90 was 64 to gentamycin 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.

P1576 The occurrence of Panton-Valentine leukocidin in MRSA strains isolated from hospitalised patients in Slovakia

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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.snars.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 cassettes 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 surgically treated deep abscess in a patient on the second hospitalisation day and the isolate should be considered a community acquired MRSA. Another PVL positive MRSA had SCCmec type III, while in other 9 PVL+ strains SCCmec cassettes were not typable. 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 linesolid.

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 typable invasive MRSA was II. All these strains were resistant also to erythromycin, clindamycin and ciprofloxacin.
4. Of all 75 analyzed MRSA strains only three were resistant to chloramphenicol. The linezolid resistance in MRSA strains from Slovak hospitals was not recorded.

P1577 Clones and toxin genes' carriage of coagulase-negative staphylococci isolated from bacteraemic infants hospitalised in an intensive care unit

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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 enterotoxins 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 (bioMérieux) 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. Biofilm production was tested by the qualitative 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. Clones were defined by PFGE of chromosomal DNA SmaI digests.

Results: In total 177/180 CNS were mecA-positive (MR-CNS). Eighty-nine strains produced biofilm, 76 of them were ica-positive, while 63 additional CNS were ica-positive and biofilm-negative. The majority of isolates (>70%) were multi-resistant including 9 strains in vitro resistant to vancomycin (MIC > 32 mg/L). Table 1 summarises the results of species and clones identification in relation to toxin genes. Three clones were dominant during the two-year period: a and b among *S. epidermidis* and h in *S. haemolyticus*. Clone c emerged in 2007. tst gene carriage was clonal related among *S. epidermidis* and *S. haemolyticus*, but enterotoxin genes were more widely distributed.

Conclusions: Multi-resistant MR-CNS clones mainly *S. epidermidis* and *S. haemolyticus* were distributed in the NICU of our University Hospital during the two-year study period. Besides biofilm formation, a factor that might contribute to their clinical importance and virulence capacity is the toxin gene carriage of the superantigens family.

Table 1. Species identification of CNS, clones and toxin genes carriage

Species (no. of strains)	Clones (no. of strains)	Positive toxin genes (no. of strains)						
		tst	*sea*	*seb*	*sec*	*sed*	*tst/sea*	*tst/sec*
S. epidermidis (106)	a (63)	11	4	–	6	1	1	2
	b (14)	–	2	–	–	–	–	–
	c (10)	1	1	–	3	–	–	1
	Others (16 clones: 19 strains)	2	1	–	3	1	–	1
S. haemolyticus (59)	h (44)	10	3	–	1	–	1	–
	Others (9 clones: 15 strains)	1	3	–	–	–	–	–
S. hominis (9)	Others (8 clones: 9 strains)	–	1	–	–	1	–	–
S. saprophyticus (1)	n	–	–	–	–	–	–	–
S. warneri (3)	3 clones	–	–	–	–	–	–	–
S. lugdunensis (2)	2 clones	–	1	–	–	–	–	–
Total: 180	43 clones	25	16	0	13	3	2	4

P1578 Clones and toxin gene profiles of methicillin-resistant *Staphylococcus aureus* causing infections among children and adults

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Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) infections and especially community-associated (CA-MRSA), including superficial, deep-seated infections and pneumonia, are widespread among adults and children in Greece. CA-MRSA usually carry the genes encoding Panton-Valentine leukocidin (PVL). We have investigated and compared the distribution and clonal evolution of CA-MRSA during 2006–2008 among adults and children in Greece.

Methods: A total of 800 MRSA were isolated from clinical specimens of infected patients from January 2006 till September 2008 in a tertiary teaching and a paediatric hospital in South Greece. Isolates were identified at species level by conventional tests, followed by the determination of MIC of oxacillin by the Etest (AB Biodisk) and their antibiotic resistance patterns to antistaphylococcal agents by the disk diffusion method. PBP2a production was investigated by a Latex agglutination test (bioMerieux). The genes: mecA (encoding PBP2a), lukS and lukF (encoding PVL), tst (encoding toxic shock syndrome toxin), sem/seg (of the enterotoxin gene cluster egc) and agr groups were defined by PCRs with specific primers. Clones were determined by PFGE of chromosomal DNA SmaI digests and MLST. CA-MRSA were isolated from patients without any predisposing risk factors.

Results: Four hundred and seventy-three MRSA were recovered from adults, and 327 from children. The majority were CA-MRSA (80% among adults and 99% from children). Genes encoding PVL were detected in 408 strains (86%) 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

the aspect of an epidemic that needs application of infection control measures.

P1579 Belgian MRSA population: nursing homes versus hospital isolates (2007)

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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% (EARSS). Considering the global and dynamic nature of MRSA, continued surveillance is important to more clearly understand the role of potential reservoirs, such as the in stream of elder 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 types 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 t038, 12% to t740 and 4% to t1359. Five 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 t038. In the hospital, 35% of the isolates belonged to the phage types group [J]*, 23% to [O]* and 39% could not be put in a defined group. Eighteen different spa types were found: 30% belonged to t740, 29% to t121, 13% to t038 and 10% to t002. 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 types groups were [O]* and [J]*; spa types t038, t740 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 types groups and dominant spa types.
Conclusion: The "super bug" MRSA has caused increasing problems in nursing homes and hospitals. Some concordances were found between the two Belgian MRSA populations, namely the same phage types groups, some spa types and few PVL positive isolates. However, typing by means of bacteriophages and spa sequences showed significant quantitative differences.

P1580 Evolution of the MRSA clones at a university hospital in the Canary Islands

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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 SmaI. 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 IIIA 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.

P1581 Community-onset methicillin-resistant *Staphylococcus aureus* in Belgium: changing epidemiology and clone diversification

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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 or in the first 48 h 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 SmaI 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-ST80-SCCmec IV (n = 47), J-ST30-SCCmec IV (n = 6) and A-ST8-SCCmec IV (n = 7). Five A-ST8-SCCmec IV strains carried the arcA 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 ST80 clone. The spread of the USA300 clone is of concern.

P1582 Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates from nursing homes (NH) of western Switzerland suggests transmission within NH

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Background: In Western Switzerland, an area of low MRSA prevalence, more than two-third 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. 07-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 shows that MRSA carriage in NH residents is not only imported from local 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

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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 (Jansen 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 catabolic 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 25ºC, 37ºC, and 45ºC 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 25ºC 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 25ºC. 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

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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%), t458 (10%), t108 (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 crossborder molecular surveillance of MRSA. The genotypic background of MRSA isolates distributed in 40 different hospitals in the Dutch and the German part of the EUREGIO 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* USA300 clone lacking arginine catabolic mobile element, in Spain

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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 ST80, but to the ST8.
Here, we report the first three Spanish isolates of the CA-MRSA USA 300 ST-8 clone without the ACME-associated arcA 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 bacteraemia (Table 1).

Case	Age	Patient location	Infection	Treatment	Risk factors	Evolution
I	6 months	Paediatrics	Impetigo on the lip	Clarithromycin 125/5 ml, susp. q12 h 10 days	–	Cure
II	4 years	Paediatrics	Breast abscess. Symptoms started twelve days before	Amoxicillin/clavulanic 2.8 ml susp. q8 h 5 days <5 cm/drainage	–	Cure
III	47 years	Emergency	Septic arthritis (ankle) and bacteraemia	Vancomycin 1 g q12 and clindamycin 600 mg q8 23 days + ciprofloxacin 750 mg q12 8 weeks	Diabetes, obesity and dilated cardiomyopathy	Cure

Results: Hybridisation patterns for the agr-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-arcA gene. In addition, all isolates contained the chp 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 gene 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

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Objectives: In the late 1990s, highly virulent Methicillin-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 SmaI 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 CA-MRSA clone USA300. Cluster analysis of SCCmec I MRSA showed 4 major clades with several established pulsotypes. 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

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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 56 MRSA strains, isolated from patients in any 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 80% 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 from only 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.

P1588 **The use of Raman spectroscopy in epidemiology of methicillin-resistant *Staphylococcus aureus***

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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 cluster 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 (8.4%) 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

Antimicrobial susceptibility of tigecycline

P1589 **Can tigecycline rescue the activity of imipenem against highly resistant metallo-β-lactamase-nonproducing *Pseudomonas aeruginosa*?**

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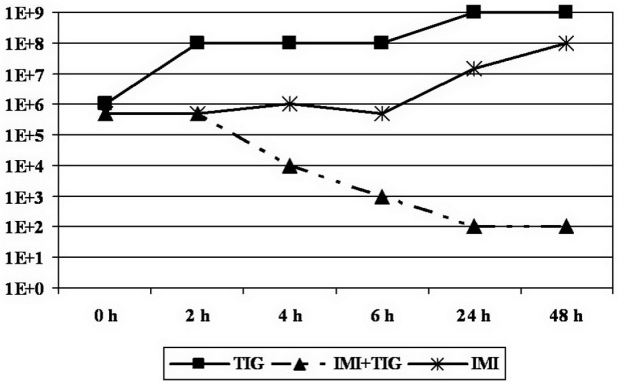
Objectives: To explore the possibility of restoring the activity of currently available antimicrobials using different combinations of these agents.
Methods: Time-kill curves of tigecycline plus imipenem were determined for one strain of
a multi-drug resistant *P. aeruginosa* in which resistance mechanisms 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 (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 $>3 \log_{10}$ units.
Conclusions: Our results point to the potential use of tigecycline in combination with carbapenems to combat multiresistance in MBL– *P. aeruginosa*.



P1590 **Activity of tigecycline against *Acinetobacter* spp. from various specimen sources collected in Europe over the past five years**

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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.

Year of isolation/ Specimen source[a]	N	TIG MIC (mg/L)				%S
		Range	Mode	MIC_{50}	MIC_{90}	
2003	40	0.12–2	1	0.5	2	80.0
2005	47	0.06–2	0.25	0.25	2	89.4
2006	87	0.06–4	1	0.5	2	88.5
2007	78	0.06–4	0.5	0.5	2	87.2
2008	20	0.06–2	1	0.5	1	95.0
Respiratory	104	0.06–4	1	0.5	2	87.5
Blood	58	0.06–2	0.25	0.5	2	89.7
Urine	55	0.06–2	0.25	0.5	2	85.5
Skin/wound	50	0.06–4	0.5	0.5	2	86.0

[a]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

to the capacity for resistance to multiple agents among AC, it is essential to continue to monitor the susceptibility patterns of this organism against new agents used to treat AC infection such as TIG.

P1591 In vitro activity of tigecycline against pathogens isolated from most common body sites – Eastern European Data – T.E.S.T. Program 2008

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Background: Tigecycline (TIG), a new glycylcycline, 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 East 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.

	Blood			Respiratory			Urinary tract		
	n	%S[a]	MIC_{90}[a]	n	%S[a]	MIC_{90}[a]	n	%S[a]	MIC_{90}[a]
EcKpKo	26	100	1	40	97.5	0.5	77	98.7	0.5
Enterobacter spp.	11	100	1	27	96.3	2	14	92.9	1
Acinetobacter spp.	9	na	–	40	na	1	6	na	–
S. aureus	17	100	0.12	25	100	0.12	7	100	–
Enterococcus spp.	12	100	0.12	5	100	–	20	100	0.12
S. pneumoniae	17	na	1	37	na	1	0		
H. influenzae	1	na	–	56	na	0.25	0		

*na = breakpoints not available.
[a] No MIC90 calculated if n < 10; %S may not be statistically significant when n's are small.

Conclusions: Tigecycline showed excellent inhibitory activity against all groups of pathogens regardless of isolation site. Tigecycline MIC90 of $\leqslant 1\,\mu g/ml$ against Gram positive pathogens (including resistant phenotypes) and MIC90 of $\leqslant 2\,\mu g/ml$ against Enterobacteriaceae and *Acinetobacter* spp. validate the potent inhibitory activity of TIG against Eastern European community/hospital pathogens.

P1592 In vitro activity of tigecycline against inpatient and outpatient pathogens from centres in Pacific Rim countries – a multi-year update

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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 (MICs) were determined by each site using supplied broth microdilution panels and interpreted according to CLSI guidelines.
Results: Selected results are listed in the tables.
Conclusion: 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 out-patient bacterial study strains. Tigecycline's inhibitory activity against Enterobacteriaceae was comparable to imipenem; vs. *Acinetobacter* spp. tigecycline's MIC90 was 32-fold lower than imipenem's. Against *S. aureus* and *Enterococcus* spp., Tigecycline's activity was similar to linezolid and vancomycin.

	Enterobacteriaceae				*Acinetobacter* spp.[a]			
	In-patients (n = 219)		Out-patients (n = 83)		In-patients (n = 37)		Out-patients (n = 7)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	98.6	1	100	1	na	0.5	na	–
Amikacin	95.2	8	96.5	4	91.9	16	100	–
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	–

*na = breakpoints not available.
[a] No MIC90 calculated if n < 10.

	S. aureus				*Enterococcus* spp.			
	In-patients (n = 61)		Out-patients (n = 19)		In-patients (n = 38)		Out-patients (n = 12)	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	100	0.5	100	0.12	100	0.12	100	0.12
Levofloxacin	75.4	4	100	0.25	71.1	>32	66.7	16
Linezolid	100	4	100	4	100	2	100	2
Minocycline	85.2	8	100	0.25	34.2	>8	16.7	8
Vancomycin	100	1	100	1	100	2	100	2

P1593 A comprehensive analysis of European data from Tigecycline Evaluation Surveillance Trials (TEST) Program, 2004–2008

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Background: Tigecycline (TIG), a new glycylcycline, 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.

Organism (n)	Tigecycline		% inhibited at MIC (μg/mL)				
	MIC_{50}	MIC_{90}	⩽0.5	1	2	4	8
A. baumannii (2053)	0.5	1	68.9	91.9	98.1	99.8	100
E. faecalis/faecium (2458)	0.12	0.12	100	–	–	–	–
Enterobacteriaceae (14311)	0.5	1	79.2	92.0	96.5	99.1	99.9
ESBLs (625)	0.5	2	63.2	83.7	93.4	98.7	100
P. aeruginosa (3394)	8	>16	1.4	2.7	6.1	19.1	56.1
S. aureus (4063)	0.12	0.25	100	–	–	–	–
S. pneumoniae (1784)	0.03	0.5	99.2	100	–	–	–
H. influenzae (2066)	0.12	0.5	96.1	99.1	100	–	–

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 vancomycin-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.

P1594 Susceptibility of Gram-negative/positive pathogens isolated in the United Kingdom and Ireland: a multi-year update

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Background: The rapid emergence of multi-drug resistant pathogens has undermined the efficacy of many widely used broad spectrum antibacterials and prompted the development of newer antimicrobials. Tigecycline is a new glycylcycline 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 at a reference laboratory. MICs were determined at each site utilising supplied broth microdilution panels and interpreted according to EUCAST guidelines.
Results: See the tables.

	E. coli, K. oxytoca/ pneumoniae, n = 346		*Acinetobacter* spp., n = 101		*P. aeruginosa*, n = 139	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	95.7	1	IE	1	–	16
Amikacin	99.4	4	86.1	64	94.2	8
Cefepime	87.6	2	–	32	82.0	32
Imipenem	100	0.5	89.1	16	88.5	8
Levofloxacin	80.6	>8	75.2	>8	63.3	>8

	S. aureus n = 162		*Enterococcus* spp. n = 94		*S. pneumoniae* n = 90	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Tigecycline	100	0.25	100	0.12	IE	0.5
Levofloxacin	77.2	8	–	>32	100	1
Linezolid	100	4	100	2	100	1
Minocycline	99.4*	0.5	54.3*	8	12.2*	2
Vancomycin	100	1	91.5	2	100	0.5

IE = There is insufficient evidence that the species in question is a good target for therapy with the drug.
– = Susceptibility testing not recommended as the species is a poor target for therapy with the drug.
*CLSI breakpoints used if no EUCAST breakpoints determined yet.

Conclusions: Tigecycline was the third most active agent against Enterobacteriaceae spp. after imipenem and amikacin, displayed the lowest MICs against *Acinetobacter* spp., but had minimal activity against *P. aeruginosa*. Against Gram-positives, tigecycline was as active as vancomycin and linezolid, and superior to levofloxacin and minocycline.

P1595 In vitro activity of tigecycline and comparators against nosocomial pathogens in Italy from 2004–2008

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Background: Tigecycline has demonstrated potent activity against a range of pathogens encountered in hospitalised patients. The TEST program was designed to determine the in vitro activity of tigecycline and comparators to nosocomial pathogens isolated in Italy between 2004–2008.
Methods: Over 1015 clinical isolates were collected/identified at each site and confirmed by the reference lab. Following CLSI guidelines, MICs were determined/interpreted using supplied broth microdilution panels.
Results: The spectrum and potency of Tigecycline against nosocomial pathogens is shown in the table.
Conclusions: Italian isolates of both Gram positive/negative hospital pathogens demonstrated excellent Tigecycline MIC90 s excluding *P. aeruginosa*. For most resistant phenotypes TIG MIC90 s were 1 mcg/mL or less and the majority of isolates inhibited at MICs of 2 mcg/mL or less. All ESBLs isolated in Italy were inhibited by Tigecycline at MICs of 2 or less. Tigecycline promises expanded broad spectrum coverage against multiply resistant pathogens isolated in Italy.

Organism (n)	Tigecycline		% inhibited at MIC				
	%S	MIC_{90}	=0.25	0.5	1	2	4
Acinetobacter spp. (68)	NA	1	53	85	99	100	–
Enterobacteriaceae (420)	94	1	40	70	91	94	99
ESBL producers[a] (45)	100	2	53	78	87	100	–
Enterobacter spp. (122)	90	2	20	56	83	90	99
E. faecalis (41)	95	0.25	100	–	–	–	–
E. faecium (29)	100	0.25	100	–	–	–	–
H. influenzae (72)	NA	0.25	90	97	100	–	–
P. aeruginosa (100)	NA	>16	–	–	–	4	14
S. aureus (MSSA) (69)	100	0.25	–	30	83	99	10
S. aureus (MRSA) (54)	96	0.5	–	7	56	93	100
S. pneumoniae (70)	NA	1	40	71	100	–	–
S. agalactiae (41)	100	0.06	100	–	–	–	–

[a] ESBL producing *E. coli*, *K. oxytoca*, *K. pneumoniae*.

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

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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.

	All Gram pos n = 179	*S. aureus* n = 76	Enterococci n = 46	*S. pneumoniae* n = 42
Tigecycline	0.5	0.25	0.25	1
Amox/Clav	2	8	1	0.12
Ampicillin	>16	>16	2	0.5
Ceftriaxone	>64	32	>64	0.12
Imipenem	1	0.5	4	0.25
Levofloxacin	8	16	>32	1
Linezolid	4	4	2	1
Minocycline	>8	0.5	>8	4
Penicillin	>8	>8	4	⩽0.06
Pip/Tazo	8	16	8	0.5
Vancomycin	2	1	4	0.5

	All Gram neg n = 369	*E. coli/Kleb* n = 141	*Enterobacter* spp. n = 66	*Acinetobacter* n = 36
Tigecycline	8	1	2	1
Amikacin	8	8	8	>64
Amox/Clav	>32	32	>32	>32
Ampicillin	>32	>32	>32	>32
Cefepime	16	4	32	32
Ceftazidime	>32	⩽8	>32	>32
Ceftriaxone	>64	16	>64	>64
Imipenem	2	0.5	2	2
Levofloxacin	>8	8	>8	>8
Minocycline	>16	16	16	1
Pip/Tazo	64	64	64	>128

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

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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 *Acinetobacter 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 Okmeydaný 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 multi-drug 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

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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 *Ralstonia* 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.

P1599 In vitro activity of tigecycline against methicillin-resistant *Staphylococcus aureus*, including animal-related strains

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Objective: To assess the in vitro activity of tigecycline of methicillin-resistant *Staphylococcus aureus*, including the recently emerging animal-related strains.
Methods: A well-defined collection of 202 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates was tested. The collection consisted of 3 subsets. The first set of isolates used in this study contained 76 hospital-associated MRSA isolates that were collected between 1990 and 1998 in The Netherlands (old MRSA). The second were 93 hospital-associated MRSA isolates collected between 2003 and 2005 (recent MRSA). The third set of isolates tested consisted of 33 animal-related MRSA strains (AR-MRSA). The MIC of tigecycline was determined by using the Etest system (AB Biodisk, Solna, Sweden). Etest strips were applied to the surface of Mueller-Hinton (MH) agar plates. The MICs of the following antimicrobial agents were determined simultaneously: gentamicin, cotrimoxazole, ciprofloxacin, erythromycin, clindamycin, rifampin, daptomycin, tetracycline, linezolid, vancomycin and teicoplanin.
Results: The MIC50 and MIC90 of tigecycline and of the other antimicrobial agents are provided in Table 1. No significant differences were found for the MIC50 of tigecycline between old MRSA, recent MRSA and AR-MRSA. The MIC values of most other antimicrobial agents decreased significantly over time. Among the 202 MRSA strains evaluated, three (1%) had a tigecycline MIC > 0.5 mg/liter, which are considered resistant. However, when Etest strips were applied on Iso-Sensitest agar plates (Oxoid Ltd.), all three isolates were susceptible for tigecycline.

Table 1. MIC_{50} and MIC_{90} of antimicrobial agents against 76 hospital-associated MRSA collected between 1990 and 1998, 93 hospital-associated MRSA collected between 2003 and 2005 and 33 animal-related MRSA strains collected in The Netherlands between 2003 and 2005

Antibiotic	Old MRSA		Recent MRSA		AR-MRSA		*P* (MIC_{90})		
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	old vs recent	old vs AR	recent vs AR
Tigecycline	0.19	0.42	0.19	0.38	0.25	0.38	0.618	0.230	0.296
Oxacillin	256	256	32.0	256	12	48	<0.001[a]	<0.001[a]	<0.001[a]
Gentamicin	12	256	0.75	48	0.38	12	<0.001[a]	<0.001[a]	<0.001[a]
Cotrimoxazole	0.13	32	0.025	0.5	0.02	0.13	<0.001[a]	<0.001[a]	0.202
Ciprofloxacin	32	32	24	32	0.38	2	<0.001[a]	<0.001[a]	<0.001[a]
Erythromycin	256	256	0.38	256	0.25	256	<0.001[a]	<0.001[a]	0.036[a]
Clindamycin	0.19	256	0.09	256	0.06	256	<0.001[a]	<0.001[a]	0.179
Rifampin	0.012	32	0.006	0.60	0.004	0.006	<0.001[a]	<0.001[a]	<0.001[a]
Daptomycin	0.38	0.75	0.38	0.75	0.13	0.19	0.243	<0.001[a]	<0.001[a]
Tetracycline	12	32	0.38	32	32	48	0.001[a]	<0.001[a]	<0.001[a]
Linezolid	1	1	1	1	0.75	1	0.032[a]	<0.001[a]	<0.001[a]
Vancomycin[b]	3	4	4	8	4	4	<0.001[a]	0.176	0.077
Teicoplanin[b]	3	12	4	12	3	4	0.385	0.05	<0.001[a]

[a] *P*-value of <0.05 is considered statistically significant.
[b] The Etest system with a large inoculum and 48 h of incubation was used.

Conclusion: The collection of MRSA showed a good susceptibility against tigecyclin and there were no differences observed in the three collections of strains. The three strains, which were resistant using MH agar plates, were susceptible when Iso-Sensitest medium was used. This discrepancy is currently under further evaluation. The other antimicrobial agents tested showed variable results. Remarkable was the trend towards a better susceptibility for non-β lactam antibiotics in more recently obtained strains. This may be caused by the emergence of MRSA in the community.

P1600 The Tigecycline Evaluation Surveillance Trial (TEST) in Canada from 2004–2008

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Objectives: Tigecycline (TIG) is a new glycylcycline with enhanced activity against many multidrug resistant (MDR) pathogens including ESBL and AmpC producing Enterobacteriaceae, methicillin-resistant *S. aureus* (MRSA), carbapenem resistant *Acinetobacter* and fluoroquinolone resistant Gram-negative rods. The TEST study evaluated the activity of TIG and comparators to pathogens in Canada 2004–2008.
Methods: A total of 1675 pathogens were collected from 8 participating sites in Canada from 2004–2008. Isolates were identified to the species level and CLSI specified MICs were performed at each site. CLSI or FDA breakpoints were used, where applicable, to determine % susceptibility.
Results: Tigecycline MICs are recorded in the table.

Organisms (n = 1675)	Tigecycline MICs (μg/ml)		
	MIC_{50}	MIC_{90}	Range
Acinetobacter spp. (n = 123)	0.12	1	0.015–2
Enterobacter spp. (n = 215)	0.5	1	0.12–8
E. coli (n = 220)	0.12	0.25	⩽0.008–2
Klebsiella spp. (n = 218)	0.5	2	0.12–8
ESBLs (n = 16)	0.12	0.5	0.06–2
Enterococcus spp. (n = 136)	0.06	0.12	0.03–0.25
VREs (n = 5)	0.03	0.06	0.03–0.06
Serratia spp. (n = 93)	0.5	1	0.25–8
H. influenzae (n = 132)	0.25	0.5	0.03–2
S. aureus (n = 214)	0.12	0.12	0.06–0.5
MRSA (n = 30)	0.12	0.12	0.06–0.5
S. agalactiae (n = 87)	0.03	0.06	⩽0.008–0.12
S. pneumoniae (n = 130)	0.03	0.03	⩽0.008–0.12

Conclusions: Tigecycline showed excellent in vitro activity against a diverse collection of pathogens isolated in Canada between 2004–2008. MIC90 values of <0.5 mcg/ml against most Enterobacteriaceae including ESBL and MIC90 of <0.12 mcg/ml against Gram-positive pathogens document the in vitro potency of tigecycline, a new glycylcycline.

P1601 Antimicrobial susceptibility of bacteraemia pathogens in Europe (2004–2008): results of the Tigecycline Evaluation Trial (TEST)

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Objectives: Bacterial resistance patterns vary by time and country. Surveillance studies help to identify those patterns to assist in therapeutic decisions. The Tigecycline Evaluation Surveillance Trial (TEST) is a multi-year global study that assists in the recognition of current trends in resistance on many levels. This report evaluates differences in susceptibility of bacteraemia pathogens isolated in Europe from 2004 to 2008.
Methods: 3762 bacteraemia pathogens were collected and identified from 2004–2008 at 77 hospitals in 21 countries in Europe. MICs for each strain were determined per EUCAST guidelines at each facility using broth microdilution.
Results: Tigecycline MICs are recorded in the table.
Conclusions: Tigecycline showed excellent in vitro activity against all causative bacteraemic pathogens with the exception of *P. aeruginosa*. Tigecycline demonstrated MIC90 values of <0.5 mcg/ml against Gram-positive pathogens (including resistant phenotypes) and MIC90 values of <2 mcg/ml against the Enterobacteriaceae including ESBL producers and *Acinetobacter* spp. Tigecycline possesses potent activity against bacteraemic pathogens.

Organisms (n = 3762)	Tigecycline MICs (μg/ml)		
	MIC_{50}	MIC_{90}	Range
Acinetobacter spp. (n = 255)	0.25	1	0.015–4
P. aeruginosa (n = 299)	8	16	0.5–>16
Enterobacter spp. (n = 481)	0.5	1	0.06–8
Enterococcus spp. (n = 356)	0.06	0.12	0.015–0.25
VREs (n = 31)	0.06	0.12	0.015–0.12
E. coli (n = 775)	0.12	0.25	0.03–2
Klebsiella spp. (n = 595)	0.5	1	0.12–8
ESBLs (n = 93)	0.5	2	0.06–8
Serratia spp. (n = 171)	0.5	1	0.12–8
H. influenzae (n = 27)	0.12	0.25	0.03–1
S. aureus (n = 432)	0.12	0.12	0.03–0.5
MRSA (n = 105)	0.12	0.25	0.03–0.5
S. agalactiae (n = 109)	0.03	0.12	0.015–0.25
S. pneumoniae (n = 289)	0.03	0.5	⩽0.008–0.5

P1602 Changes in susceptibility of select nonfermentors in Europe: 2004–2008

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Background: Tigecycline was approved for use in Europe in 2006 for complicated skin and soft tissue infections and has demonstrated promising activity against multiply-resistant species and phenotypes. 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.

	MIC_{90} (μg/mL)									
	Acinetobacter spp.					*Pseudomonas aeruginosa*				
	2004	2005	2006	2007	2008	2004	2005	2006	2007	2008
n	455	232	572	602	575	609	364	810	890	575
Tigecycline	1	1	1	1	1	>16	>16	>16	>16	>16
Amikacin	>64	64	>64	>64	>64	16	16	16	16	16
Cefepime	32	32	>32	>32	32	32	32	32	32	32
Ceftazidime	>32	>32	>32	>32	>32	32	32	>32	32	>32
Ceftriaxone	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Meropenem	na	>16	16	16	16	>16	>16	>16	16	16
Levofloxacin	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8
Minocycline	8	1	2	2	2	>16	>16	>16	>16	>16
Pip/tazo	>128	>128	>128	>128	>128	128	64	128	128	128

Conclusions: Tigecycline demonstrated no shift in MIC values in Europe over four years from its pre-marketing baseline values when tested against non-fermentors. Consistent MIC90 values 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.

P1603 Is the number of ESBLs increasing in Asia/Pacific Rim countries? A longitudinal evaluation of organisms associated with ESBL production between 2004–2008

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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.

Country	2004		2005		2006		2007		2008	
	%ESBL	MIC_{90}	%ESBL	MIC_{90}	%ESBL	MIC_{90}	%ESBL	MIC_{90}	%ESBL	MIC_{90}
Australia	0	NA	1.46	1	3.1	4	2.9	1	3.0	1
China	18	1	34.8	8	24.1	0.25	NA	NA	NA	NA
Hong Kong	NA	NA	0	NA	0	NA	7.2	0.5	NA	NA
India	65.3	1	NA	NA	0	NA	12.6	1	10.5	1
Korea	NA	NA	26.5	1	6.2	2	18.5	1	24.1	1
Pakistan	69.2	4	17.6	2	0	NA	NA	NA	NA	NA
Philippines	2.1	0.5	NA	NA	9.8	2	9.1	0.5	8.8	0.5
Singapore	18.4	1	0	NA	27.1	0.5	9.2	2	NA	NA
Taiwan	NA	NA	NA	NA	18.3	2	24.1	1	22.2	2

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.

P1604 Activity of tigecycline and five comparators against recent Gram-positive anaerobes in Europe – the Tigecycline European Surveillance Trial

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Objectives: Tigecycline is a novel antimicrobial with expanded broad-spectrum activity from a new class of compounds, the glycylcyclines. Tigecycline was developed to provide activity against tetracycline and multi-drug-resistant Gram-positive pathogens and has demonstrated significant broad-spectrum activity against aerobic and anaerobic Gram-positive and Gram-negative microorganisms. In this study we evaluated tigecycline and five comparator compounds against European Gram-positive anaerobic isolates.
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.

	Anaerococcus spp. (n = 43)		*Clostridium* spp. (n = 249)		*Fingoldia magna* (n = 56)		*Peptoniphilus* (n = 28)		PStrep[a] (n = 78)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Tigecycline	<0.06	0.12	<0.06	0.5	<0.06	0.12	<0.06	<0.06	<0.06	0.12
Clindamycin	<0.25	>8	2	>8	0.5	>8	<0.25	>8	<0.25	<0.25
Metronidazole	0.25	1	0.5	1	0.25	0.5	0.5	1	0.25	0.5
Pip/Tazo	<0.06	0.25	0.5	8	<0.06	0.12	<0.06	<0.06	<0.06	0.25
Meropenem	<0.06	0.12	0.12	1	<0.06	0.12	<0.06	<0.06	<0.06	0.25
Penicillin	<0.25	0.5	<0.25	2	<0.25	0.5	<0.25	<0.25	<0.25	0.5

[a] PStrep, *Peptostreptococcus* spp.

Conclusions: Tigecycline showed excellent in vitro activity against Gram-positive anaerobic microorganisms isolated from European hospitals. MIC50 values for tigecycline against all isolates tested were <0.06 mcg/mL, while MIC90 values were <0.5 mcg/mL. Using the FDA breakpoint of <4 mcg/mL, 100% of the isolates were susceptible to tigecycline, including difficult to treat *Clostridium difficile*.

P1605 Activity of tigecycline against recent European clinical isolates of *Clostridium difficile*

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Background: The role of toxin-producing *Clostridium difficile* in antibiotic-associated diarrhoea is well established. The incidence of this difficult to treat disease has increased dramatically since the 1980s. Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections, including Gram-positive anaerobes. The T.E.S.T. program determined the in vitro activity of tigecycline and five comparators against *Clostridium difficile* strains collected from 18 investigational sites in 6 countries in Europe throughout 2008.
Methods: A total of 88 clinical isolates of *C. difficile* 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.
Results: Results are shown in the table.

	Clostridium difficile (n = 88)			
	MIC_{50}	MIC_{90}	%S	Range
Tigecycline	⩽0.06	0.25	100	⩽0.06–2
Clindamycin	4	>8	31.8	<0.25–>8
Meropenem	1	2	100	⩽0.06–4
Metronidazole	0.5	1	100	⩽0.12–2
Penicillin	1	4	14.8	⩽0.25–>32
Pip/tazo	4	16	98.9	<0.06–>64

Conclusions: Tigecycline showed excellent in vitro activity against European clinical isolates of *Clostridium difficile*, with MIC50/MIC90 values of <0.06 and 0.25 mcg/mL. Tigecycline's low MIC50/MIC90 values suggest that it may be an option against this difficult to treat pathogen.

P1606 In vitro susceptibility to tigecycline and to an extended panel of antimicrobials of Gram-positive and Gram-negative isolates in Portugal

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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

antimicrobials of a collection of clinical isolates isolated in Portugal in 2007.
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, cefoxitine, erythromycin, 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, piperacillin-tazobactam, cefepime, ceftazidime, ampicillin-sulbactam, trimetoprim-sulfamethoxazole, minocyclin, amoxicillin-clavulanate, cefotaxime, gentamicin, amikacin, ampicillin, ciprofloxacin and tetracycline.
Results: Among Gram positive isolates (n = 839), all *Staphylococcus* spp. isolates (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 = 232) 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 Acinectobacter 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

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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 '06-'08. Isolates were tested centrally 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) and ⩾4-fold more potent than cefepime (4; 93.2%S), ceftriaxone (64; 83.9%S), ciprofloxacin (>4; 80.5%S), and piperacillin-tazobactam (64; 85.2%S). Against EC, KP, CS, ES, and SM the TIG MIC90 s (mg/L) were, 0.5, 1, 0.5, 2, and 1, respectively. Overall TIG %S ranged 89.9% (ES) to >99% (EC and CS). The TIG MIC50/MIC90 was not notably affected by R to advanced generation cephalosporins, though the %S for ES and SM was lower among resistant isolates relative to susceptible isolates. Isolates non-susceptible to minocycline remained largely susceptible to TIG regardless of species. TIG MIC profiles and %S were similar across evaluated countries for EC (99–100%S), KP (93–98.6%S), and CS (100%S). Among ES and SM decreased S was noted for isolates from the UK (70.6% SM, 81.6% ES) and to a lesser extent with Spain (92.3% SM, 84.6% ES) and Czech Republic (84.2% SM, 90.6% ES), while isolates in other countries were more susceptible (90–100%S).
Conclusions: Overall, EN in EU was 96.4% S to TIG. Isolates with clinically relevant R remained susceptible to TIG, though for ES and SM there was a drop in TIG %S among isolates resistant to advanced generation cephalosporins. TIG activity was consistent across the evaluated countries, though low %S for ES and SM was noted among UK isolates. Increased use of TIG and the concern regarding R among EN highlight the continued need for surveillance both on the regional and local levels.

P1608 Update on the spectrum and potency of tigecycline tested against 7,133 Gram-positive and -negative pathogens from 13 countries (2008)

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Objectives: To assess the contemporary potency and spectrum of tigecycline against Gram-positive (GP) and -negative (GN) pathogens, including strains with various resistant phenotypes. Tigecycline is a glycylcycline antimicrobial recently approved by the European Medicines Agency (EMEA) for the treatment of complicated skin and skin structure as well as intra-abdominal infections.
Methods: A total of 7,133 GN and GP clinically-significant non-duplicate isolates from multiple types of infections were collected from 12 EU countries and Israel that participated in tigecycline surveillance program during 2008. Susceptibility (S) testing was performed by a central monitoring laboratory (JMI Laboratories) using CLSI methods (M7-A7, 2006). All concurrent quality control tests were within published ranges.
Results: Tigecycline inhibited all *S. aureus* and coagulase-negative staphylococci (CoNS) isolates at ⩽0.5 mg/L, regardless of S to oxacillin, with a MIC90 at 0.25 mg/L. Tigecycline also had potent activity against all enterococci (MIC90, 0.25 mg/L), including vancomycin-resistant (VRE) strains, and β-haemolytic streptococci (βHS, MIC90, 0.06 mg/L). The MIC90 for Enterobacteriaceae was 0.25, 0.5 and 1 mg/L for *E. coli*, *Klebsiella* spp. and *Enterobacter* spp., respectively. Tigecycline was active against *Acinetobacter* spp. (MIC90, 2 mg/L), but less active against isolates of *P. aeruginosa* (MIC50/90, 4/>4 mg/L). Tigecycline activity is summarised in the Table against nine organism groups.

Organism (no. tested)	TIG MIC (mg/L)		Cum. % inhibited at Tigecycline MIC (mg/L):							
	50%	90%	⩽0.03	0.06	0.12	0.25	0.5	1	2	4
S. aureus (2,505)	0.12	0.25	0.2	22.9	70.0	99.2	100.0	–	–	–
CoNS (648)	0.12	0.25	2.2	24.9	59.1	94.3	100.0	–	–	–
Enterococcus spp. (898)	0.12	0.25	9.4	39.0	74.3	100.0	–	–	–	–
βHS (323)	⩽0.03	0.06	77.1	96.9	99.7	100.0	–	–	–	–
E. coli (1,262)	0.12	0.25	0.0	16.6	66.6	95.3	99.6	100.0	–	–
Klebsiella spp. (529)	0.25	0.5	0.0	0.0	11.2	68.6	90.7	96.8	99.8	–
Enterobacter spp. (281)	0.25	1	0.0	0.0	4.6	53.7	87.2	96.4	99.3	100.0
P. aeruginosa (517)	4	>4	0.0	0.0	0.0	0.0	0.8	2.5	12.0	52.6
Acinetobacter spp. (170)	1	2	0.0	2.9	11.2	24.1	38.2	75.5	94.1	98.8

Conclusions: 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 Enterobacteriacea, including those producing broad-spectrum β-lactamases such 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

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Objectives: To assess the contemporary potency and spectrum of tigecycline tested against Gram-positive and -negative CARTI pathogens,

including strains with various resistant (R) phenotypes. Tigecycline is a glycylcycline 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.

Organism (no. tested)	TIG MIC (mg/L)		Cum. % inhibited at Tigecycline MIC (mg/L):						
	50%	90%	⩽0.03	0.06	0.12	0.25	0.5	1	2
S. aureus (714)	0.12	0.25	0.6	27.6	80.1	99.4	100.0	–	–
Oxacillin-S (450)	0.12	0.25	0.7	28.4	80.4	99.8	100.0	–	–
Oxacillin-R (264)	0.12	0.25	0.4	26.1	79.6	98.9	100.0	–	–
S. pneumoniae (5,375)	⩽0.03	0.06	80.3	96.3	99.3	99.9	>99.9	100.0	
Penicillin-S (3,519)	⩽0.03	0.06	81.7	96.5	99.4	>99.9	–	–	–
Penicillin-I (881)	⩽0.03	0.06	83.9	96.8	99.6	100.0	–	–	–
Penicillin-R (975)	⩽0.03	0.06	72.1	95.1	98.7	99.8	99.9	100.0	–
H. influenzae (3,129)	0.5	1	0.1	0.1	0.2	11.7	73.7	99.6	100.0
β-lactamase-neg. (2,486)	0.5	1	0.1	0.1	0.2	12.4	74.1	99.6	100.0
β-lactamase-pos. (642)	0.5	1	0.3	0.3	0.5	9.0	72.0	99.7	100.0
M. catarrhalis (17)	0.12	0.12	23.5	47.1	100.0	–	–	–	–

Tigecycline inhibited *S. aureus* at ⩽0.5 mg/L, with a MIC90 at 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. catarrhalis* (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 penicillin-R *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 TEST Program in 2008

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Background: Tigecycline (TIG), a new glycylcycline, 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 Gram positive/negative species. Isolates for the overall T.E.S.T program were collected from 1016 hospital sites in 53 countries from 2004 to 2008.

Methods: A total of 5,084 clinically significant respiratory isolates collected worldwide were analyzed in this survey. The isolates were identified to the species level at the participating sites and confirmed by the central laboratory. MICs were determined by each site using supplied broth microdilution panels and interpreted according to 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.

	H. influenzae (2,433)				*S. pneumoniae* (2,651)			
	%SUS	%INT	%RES	MIC_{90}	%SUS	%INT	%RES	MIC_{90}
Tigecycline	na	na	na	0.5	na	na	na	0.5
Ceftriaxone	99.8	–	0.2	=0.06	97.8	1.3	0.9	1
Levofloxacin	100	–	–	0.03	99.9	0.1	–	1
Minocycline	na	na	na	1	na	na	na	4
Amox/Clav	99.8	–	0.2	1	94.8	3.2	2.0	2
Penicillin	–	–	–	–	61.0	26.8	11.3	2
PipTazo	99.8	–	0.2	=0.06	na	na	na	2
Linezolid	–	–	–	–	100	–	–	1
Vancomycin	–	–	–	–	100	–	–	0.5

*na = breakpoints not available.

P1611 Evaluation of in vitro activity of tigecycline and ten comparators against methicillin-resistant *Staphylococcus aureus* from Europe: TEST Program 2004–2008

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Background: Tigecycline (TIG), a member of a new class of antimicrobials (glycylcyclines), 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, 100, and 83.7, respectively. MIC50/90 (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

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Objectives: Tigecycline has been shown to be active against a wide range of bacteria including multi-resistant pathogens. The German Tigecycline Evaluation Trial (G-TEST) 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

2005 (n = 523) and 2007 (n = 473) were included. Agents tested were tigecycline, ciprofloxacin, cefotaxime, gentamicin, imipenem, linezolid, moxifloxacin, oxacillin, piperacillin-tazobactam, and vancomycin. 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.

Results: The majority of isolates derived from respiratory tract specimens (n = 363, 36%), followed by blood cultures (n = 245, 25%) and wound specimens (n = 205, 21%). Of the *S. epidermidis* isolates, 84% were oxacillin-resistent. Rates of resistance to moxifloxacin and gentamicin were 14% and 9% for MSSA, 92% and 17% for MRSA, and 44% and 63% for *S. epidermidis*. All staphylococci were susceptible to vancomycin and linezolid. In contrast, 12% of the *E. faecium* isolates were resistant to vancomycin. High-level resistance to gentamicin was >40% in both enterococcal species. Tigecycline inhibited all Gram-positive cocci at ⩽0.5 mg/l. MIC50/90 values of tigecycline for *E. cloacae*, *E. coli* and *K. pneumoniae* were 0.5/1, ⩽0.125/0.25 and 0.5/4 mg/l, respectively. Rates of resistance (%) to tigecycline, ciprofloxacin, cefotaxime, gentamicin, imipenem and piperacillin-tazobactam were as follows: *E. cloacae* – 6, 5, 51, 5, 0, 25; *E. coli* – 0, 26, 11, 12, 0, 6; *K. pneumoniae* – 12, 18, 12, 7, 0, 12. Of the *E. coli* and *K. pneumoniae* isolates, 22 (12%) and 16 (13%) were ESBL+. Tigecycline inhibited 36/38 (95%) ESBL+ isolates at ⩽2 mg/l.

Conclusions: Tigecycline demonstrated excellent in vitro activity against the tested Gram-positive and Gram-negative pathogens including MRSA, VRE and ESBL producing bacteria. Therefore, tigecycline may be an alternative for the treatment of infections caused by pathogens of the tested species on intensive care units.

P1613 Profile of tigecycline and comparator agents against Gram-positive organisms collected across the United States and Europe in 2007–2008

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Objective: Tigecycline (TIG), a broad-spectrum glycylcycline, was approved in both the US and Europe (EU) for treatment of complicated skin and intra-abdominal infections, and is currently undergoing development for the treatment of community-acquired pneumonia. Gram-positive cocci including *Staphylococcus aureus* (SA), *Streptococcus pneumoniae* (SP), *S. pyogenes* (GAS), and enterococci are significant pathogens associated with these indications. It is important to understand the activity profile of new agents against target organisms and to monitor for development of resistance. This study examines the activity profile of TIG and other select agents against recent isolates of Gram-positive pathogens collected across the US and EU.

Organism	Agent	US				EU			
		Total n	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S	Total n	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S
SA	Tigecycline	1807	0.12	0.25	100	428	0.12	0.25	100
	Clindamycin		0.12	>2	80.3		0.12	>2	73.1
	Daptomycin		0.5	1	99.9		0.5	1	99.8
	Linezolid		1	2	100		1	2	100
	Vancomycin		1	1	100		1	1	100
SP	Tigecycline	250	0.015	0.03	–[a]	138	0.015	0.03	–
	Clindamycin		0.06	>2	84.4		0.06	>2	81.2
	Daptomycin		0.12	0.25	–		0.12	0.25	–
	Linezolid		0.5	1	100		0.5	1	100
	Vancomycin		0.25	0.5	100		0.25	0.5	100
GAS	Tigecycline	250	0.03	0.06	100	100	0.03	0.03	100
	Clindamycin		0.06	0.06	98.0		0.06	0.06	98.0
	Daptomycin		0.06	0.06	100		0.06	0.06	100
	Linezolid		1	1	100		1	1	100
	Vancomycin		0.25	0.25	100		0.25	0.25	100
ES	Tigecycline[b]	100	0.12	0.5	80.0	25	0.12	0.25	96.0
	Clindamycin		>2	>2	–		>2	>2	–
	Daptomycin		1	2	99.0		2	4	96.0
	Linezolid		0.5	1	100		1	2	100
	Vancomycin		1	2	96.0		1	2	96.0

[a] CLSI and EUCAST breakpoints are unavailable for interpretation of susceptible (S).

[b] *E. faecalis* (vancomycin-susceptible isolates only) FDA breakpoints were applied to all US ES.

Methods: In 2007–2008 a total of 1,807 SA, 250 SP, 250 GAS, and 100 *Enterococcus* spp. (ES; 100 *E. faecalis*) clinical isolates from the US and 428 SA, 138 SP, 100 GAS, and 25 ES (18 *E. faecalis*; 7 *E. faecium*) from EU, were tested centrally by broth microdilution (CLSI M7-A7). EUCAST and FDA breakpoints were used to interpret EU and US TIG MIC results, respectively, and CLSI (M100-S18) breakpoints were used to interpret all other agents.

Results: See the table.

Conclusions: TIG had potent in vitro activity against all evaluated Gram-positive organisms, with identical or lower MIC90 s than comparator agents. Isolates of SA and GAS were 100%S to TIG, while susceptibility of ES varied from 80% in the US to 96%S in EU. The activity profile of TIG was consistent between EU and US isolates of the evaluated organisms. Further surveillance is warranted both to support the ongoing development of TIG and to monitor for the emergence of resistance.

P1614 In vitro activity of tigecycline against pathogens from Crete, Greece – T.E.S.T Program 2006

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Objective: Tigecycline (TIG), a member of glycycyclines, 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: Tigecycline activity against aerobic Gram-positive and negative bacteria is shown in the table.

Organism (n)	MIC_{50}	MIC_{90}	Range	% susceptibility
S. aureus (25)	0.12	0.12	0.06–0.25	100
Enterococcus spp. (15)	0.06	0.25	0.03–0.25	100
S. pneumoniae (15)	1	1	0.25–2	na*
S. agalactiae (10)	2	2	2–2	0
E. coli (25)	0.12	0.12	0.06–0.5	100
Klebsiella spp. (25)	0.5	2	0.12–16	88
Enterobacter spp. (25)	0.5	0.5	0.25–2	96
Serratia spp. (10)	1	2	0.25–4	80
H. influenzae (15)	0.12	0.25	0.06–0.25	na
Acinetobacter spp. (15)	0.5	2	0.06–4	na
P. aeruginosa (20)	8	16	4–16	na

*na: not applicable.

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.

P1615 Survival predictors in patients treated with tygecicline in clinical practice

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Background: Most clinical experience with Tigecycline (TGC) proceeds from clinical trials. The objective of the study was to determinate survival predictor factors in patients treated with TGC.

Methods: Prospective observational study of all patients treated with TGC from april 2007-april 2008 admitted in a 450-bed university hospital.

Measures: demographics, length of stay (LOS), ICU admission, SAPS-II at admission (SAPSII-A) and at TGC beginning (SAPSII-TGC), empirical TGC-therapy, TGC days, patients with previous (PA) and concomitant antibiotics (CA), isolated microorganisms before TGC-treatment (M-TGC), infection site, microbiological eradication (ME), favourable clinical outcome (FCO) and mortality during or 28 days after finishing TGC therapy (M). SAPS-II was transformed in a dichotomic variable (cut-off point 40). Fischer exact test for dichotomic and "U" Mann-Whitney test for continuous variables and a multiple logistic regression for prediction survival were employed.
Results: Patients: 62; male: 32 (51.6%); mean age: 61.6 (SD+15.3); mean LOS: 39.9 (SD+34.1); ICU patients: 24 (38.7%); SAPSII-A: 31.3 (SD+10.4); SAPSII-TGC: 31.2 (SD+11.7), empirical treatment 12 (19.4%); TGC days: 7.5 (SD+5.7); PA: 41 (66.1%); CA: 43 (69.4%); infection site: SSTI 21 (33.9%), intraabdominal 19 (30.6%), low respiratory tract 11 (17.7%), CNS 5 (8.1%), others (9.7%); main M-TGC: *Stenotrophomonas maltophilia* 9 (14.50%), *Escherichia coli* 8 (12.9%); FCO: 35 (58.3%), ME 29 (47.5%) and M: 11 (17.7%).
In multivariate analysis, only the lack of isolation of *S. maltophilia* [OR: 23.1 (CI95%: 3.3–160.5) (p = 0.001)], and SAPS-II-TGC <40 [OR: 13.2 (CI95%:2.1–82.2) (p = 0.006)] were independently associated with survival.
Conclusions:
- Survival predictor factors in patients treated with TGC were a SAPSII-TGC score lower than 40 and lack of *S. maltophilia* isolation.
- TGC treatment was related to a low mortality considering the high severity of patients.

P1616 Activity of tigecycline against multidrug-resistant anaerobic isolates from Europe in 2008 – the T.E.S.T. Program

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Background: Tigecycline, a member of a new class of antimicrobials (glycylcyclines), 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 MIC90 s of tigecycline to MDR groups 0–2 are shown in the table.

	MDR Group MIC_{90} (n)		
	Group 0	Group 1	Group 2
Aerococcus spp.	0.12 (33)	0.12 (10)	–
Bacteroides spp.	1 (354)	4 (104)	2 (8)
Clostridium spp.	0.5 (167)	1 (60)	1 (22)
Finegoldia spp.	0.12 (44)	⩽0.06 (12)	–
Peptoniphilus spp.	⩽0.06 (24)	⩽0.06 (4)	–
Peptostreptococcus spp.	<0.06 (72)	0.12 (6)	–
Prevotella spp.	0.5 (130)	0.5 (37)	0.12 (1)

*MDR Group is defined as resistant to 0, 1, or 2 or more separate drug classes.

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.

Antibacterial susceptibility of Gram-positives

P1617 Comparative in vitro activity of moxifloxacin, tigecycline and 8 other antimicrobial agents against contemporary clinical isolates of *Streptococcus pyogenes*

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Objectives: *Streptococcus pyogenes* is the predominant cause of bacterial pharyngitis and is often associated with skin and skin structure infections. We tested contemporary clinical isolates for susceptibility resistance to 10 antimicrobial agents: azithromycin (AZ), ciprofloxacin (CP), clarithromycin (CL), erythromycin (ER), gemifloxacin (GM), levofloxacin (LVF), moxifloxacin (MFX), penicilllin (P), telithromycin (TEL) and tigecycline (TIG).
Methods: Minimum inhibitory concentration (MIC) testing was by microbroth dilution according to the Clinical and Laboratory Standards Institute recommended procedure. Briefly, 10^5 cfu/ml of test organism in Mueller-Hinton broth were exposed to doubling drug dilutions and following incubation under ambient conditions (35–37 degree Celsius in O2 for 18–24 hours) the lowest concentration preventing growth was the MIC.
Results: A total of 177 clinical isolates were tested. MIC50, MIC90 and MIC range values (mg/L) for AZ, CIP, CL, ER, GEM, LFX, MFX, P, TEL and TIG respectively were as follows: 0.125, 0.125, 0.063–⩾4; 0.5, 0.5, 0.125–4; 0.031, 0.031, 0.004–2; 0.031, 0.031, ⩽0.008–2; 0.008, 0.016, ⩽0.004–0.063; 0.5, 0.5, 0.5–2; 0.125, 0.125, 0.031–0.5; 0.008, 0.008, ⩽0.002–0.016; 0.008, 0.016, ⩽0.002–0.016; 0.016, 0.031, ⩽0.004–0.063. Two percent of strains were macrolide resistant.
Conclusions: Macrolide resistance rates were low (2%) against *S. pyogenes* strains. Of the quinolones, GEM and MFX were 4–32 more active than CIP or LFX. TIG and TEL MIC90 values were equal to or lower than those for AZ, CL, ER. No strains were resistant to penicillin and the highest MIC was 0.016 mg/L. CIP and LFX were the least active agents based on MIC90 values (0.5 mg/L). The rank order of potency based on MIC90 values were P > TEL = GEM > TIG = CL, ER > MFX = AZ > LFX = CFX.

P1618 A multi-centre evaluation of in vitro activity of oritavancin and comparators against staphylococci, enterococci, and streptococci – the ORION Study

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Background: Oritavancin (ORI) is a bactericidal lipoglycopeptide with multiple modes of action, and has superior pharmacodynamic properties compared to vancomycin. The ORI Susceptibility Profile Initiative (ORION) study determined the activity of ORI against a variety of Gram-positive pathogens collected in the USA and South Korea. This analysis focuses on the in vitro activity of ORI and comparators against common clinical staphylococcal, streptococcal, and enterococcal species and resistant phenotypes.
Methods: 2,973 clinical isolates were collected from 35 labs in the USA (21) and Korea (14) in 2007–2008. MICs were determined using broth microdilution according to CLSI guidelines and interpretive criteria (M7-A7 and M100-S18).
Results: ORI MIC90 (mg/L) and % Susceptible (%S) compared to vancomycin (VAN), daptomycin (DAP) and linezolid (LIN) are presented in the table.
Conclusions: ORI had the lowest MICs against most clinical isolates compared to VAN, DAP, and LIN. Against all species and phenotypes, ORI MIC90 s were 2- to >1024-fold lower than comparators, except for DAP vs. *S. pyogenes*, where DAP and ORI MIC90 s were both 0.25 mg/L.

Notably, ORI demonstrated potent in vitro activity against both pan-sensitive isolates and those resistant to VAN, DAP, or LIN.

Organism or phenotype (n)	ORI		VAN		DAP		LIN	
	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S
S. pneumoniae (125)	0.015	na*	0.5	100	0.5	na	2	100
Penicillin-RES (46)	0.008	na	0.5	100	0.5	na	2	100
Macrolide-RES (64)	0.008	na	0.5	100	0.5	na	2	100
S. agalactiae (127)	0.25	na	1	100	0.5	100	2	100
S. pyogenes (118)	0.25	na	0.5	100	0.25	100	2	100
Viridans group strep (104)	0.06	na	1	99.0	1	96.2	2	100
S. aureus (1466)	0.25	na	1	99.9	1	99.9	4	100
MRSA (678)	0.25	na	2	99.9	1	99.7	4	100
MSSA (788)	0.25	na	1	100	0.5	100	4	100
Coag-neg Staph (160)	0.12	na	2	100	1	98.8	2	98.1
E. faecalis (455)	0.12	na	4	95.6	2	100	2	96.9
VAN-RES (18)	0.25	na	>128	0	2	100	2	100
E. faecium (393)	0.12	na	>128	46.1	4	99.0	4	89.6
VAN-RES (212)	0.12	na	>128	0	4	99.1	4	85.8

*na = breakpoints not defined.

P1619 Activity of telavancin against reference and recent clinical isolates of hVISA and VISA assessed by population analysis profiling

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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.

P1620 Longitudinal analysis of the in vitro activity profile of oritavancin and comparator glycopeptides against Gram-positive organisms from Europe: 2005–2008

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Objective: Oritavancin (ORI), currently in clinical development for use in the treatment of infections caused by Gram-positive (GP) bacteria, is a potent bactericidal lipoglycopeptide. ORI has previously shown potent activity against GP organisms, including resistant (R) strains. This comparative analysis was undertaken to profile the in vitro activity of ORI in Europe (EU) across multiple years.
Methods: In total 2036, recent (2005–2008) clinical isolates of *S. aureus* (SA; n = 757), coagulase-negative staphylococci (CoNS; n = 340), *Enterococcus* spp. (EN; n = 492), *S. pyogenes* (GAS; n = 235), and *S. agalactiae* (GBS; n = 212) from 49 hospital sites in EU (15 countries) were centrally tested by broth microdilution (CLSI; M7-A7) against ORI, teicoplanin (TEI), and vancomycin (VAN). ORI assays included 0.002% polysorbate-80 throughout.
Results: ORI MIC90 s ranged from 0.12–0.25 mg/L for SA across all years studied; MIC90 s for VAN (1 mg/L) and TEI (0.5–2 mg/L) were 4–16 fold higher, comparatively. For CoNS, ORI MIC90 s ranged from 0.12–0.25 mg/L across all years studied; MIC90 s for VAN (2 mg/L) and TEI (2 to 8 mg/L) were 16–64 fold higher, comparatively. ORI modal MICs, MIC50 s, and MIC90 s by year were generally equivalent (⩽1 doubling dilution) against oxacillin (OX)-susceptible (S) and -R staphylococci (STA). The ORI MIC90 for VAN-S EN was 0.06 mg/L from 2005 to 2008. For the VAN-S population MIC90 s for TEI (0.25–0.5 mg/L) and VAN (2 mg/L) were consistently higher compared with ORI each year. For VAN-non S EN (2005 only), ORI MIC90 was 0.5 mg/L compared to 256 mg/L and >256 mg/L for TEI and VAN, respectively. ORI MIC90 s ranged from 0.12 to 0.25 mg/L against GAS and 0.25–0.5 for GBS, including both erythromycin-S and -R isolates, similar to the MIC90 s of VAN (GAS: 0.25 mg/L; GBS: 0.5 mg/L) and slightly higher than those of TEI (GAS: ⩽0.03 mg/L; GBS: 0.12 mg/L).
Conclusions: No trend towards decreased susceptibility or elevated MICs was apparent for ORI or comparator agents over time against the evaluated GP cocci. ORI was more potent than comparator glycopeptides against both STA and EN, including resistant isolates, across the evaluated years.

P1621 Susceptibility of coagulase-negative staphylococci to telavancin, a new lipoglycopeptide antibiotic and comparison with vancomycin

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Objectives: Coagulase negative staphylococci (CoNS) have become recognized as important human pathogens. Many CoNS are resistant to methicillin and require treatment with vancomycin. In recent years reduced susceptibility to vancomycin has been noted amongst *Staphylococcus aureus* and to a lesser extent among CoNS. Telavancin is a new lipoglycopeptide which has shown increased activity against Gram positive organisms compared to vancomycin. We undertook this study to compare in vitro susceptibilities of a large series of speciated coagulase negative staphylococci (CoNS) to telavancin and vancomycin, using agar dilution. Further, the study assessed the reliability of the Vitek® 2 system for determining vancomycin susceptibility.
Methods: Susceptibility was performed on 699 non duplicate CoNS isolates, comprising 15 species and subspecies. Agar dilution was performed using methodology described by the CLSI and was used to determine MICs to telavancin and vancomycin. All strains were also tested for their susceptibility to vancomycin by the Vitek® 2 system, using the AST-GP66 card.
Results: Of 669 strains, eight strains of *S. cohnii* cohnii failed to grow in the Vitek® 2 system. Overall telavacin was more active than vancomycin. For most species and subspecies of CoNS the MIC's of telavancin were

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 (range, <0.125 to 0.5 mg/L) and 0.5 mg/L (range, <0.125 to 1.0 mg/L) respectively, whereas the overall MIC 50 and MIC 90 for vancomycin by the agar dilution method were 1.0 (range 0.5 to 1.0 mg/L) and 2.0 mg/L (range, 0.5 to 2.0 mg/L). Not all MICs obtained on the Vitek® 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® 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® 2 system.
Conclusion: Telavancin is more active against CoNS than vancomycin, with MICs 1 to 2 dilutions lower. For evaluable isolates the Vitek® 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

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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 MIC90 values of 0.25 or 0.5 mg/L for each country. CPT was active against both methicillin-susceptible SA and MRSA (MIC90 s of 0.25 and 1 mg/L, respectively). All SP and other β-haemolytic streptococci (BHS) were inhibited by CPT at ⩽0.015 mg/L. CPT was also active against *Enterococcus faecalis* and *Escherichia coli* with MIC90 s of 1 and 0.5 mg/L, respectively. CPT was less active against *Klebsiella pneumoniae* (MIC90, >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.

P1623 In vitro susceptibility profiles of teicoplanin, vancomycin, linezolid and daptomycin among coagulase-negative staphylococci in oncology patients with bacteraemia

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Objectives: To determine the in vitro susceptibility profiles of teicoplanin, vancomycin, linezolid and daptomycin among coagulase-negative staphylococci (CNS) isolated from bacteraemic patients in a specialist oncology hospital, and whether any significant change in minimum inhibitory concentration (MIC) over the study period had occurred.
Methodology: 302 isolates of clinically significant CNS from bacteraemic patients collected between the years 2001 and 2007 were examined retrospectively and the MIC to teicoplanin, vancomycin, linezolid and daptomycin determined by Etest methodology. Results were expressed as geometric mean MICs and frequency distributions according to species, source of infection and clinical unit. One-way analysis of variance was used to determine whether any significant change in the mean MIC had occurred over the study period.
Results: The overall prevalence of decreased susceptibility to teicoplanin was considered low, when compared to similar studies, at 5.3%; however, there was a peak in 2006 at 19.1%. No decreased susceptibility to vancomycin, linezolid or daptomycin was detected. Using one-way analysis of variance, a significant increasing linear trend was detected in teicoplanin MICs over time ($p < 0.05$); conversely, vancomycin and linezolid MICs decreased linearly ($p < 0.05$). No linear trend was detected in daptomycin MICs. The percentage of isolates with a teicoplanin MIC of greater than 2 mg/L increased between 2001 and 2007, with nearly 50% of isolates having an MIC of greater than 2 mg/L in 2006. Decreased susceptibility to teicoplanin was nearly three times more prevalent in isolates of *S. haemolyticus* (16%) than in *S. epidermidis* (5.5%); all other species were fully susceptible. Isolates attributed to Hickman line-related bacteraemia had a higher rate of decreased susceptibility than isolates believed to be attributed to febrile neutropaenia (8.1% compared to 3.4%). Although cases of bacteraemia caused by CNS were most common in patients admitted to the paediatric unit (40.7%), patients admitted to the leukaemia unit had a higher rate of bacteraemia with isolates showing decreased susceptibility to teicoplanin (10.8% compared to 4.1% in paediatrics).
Conclusion: The activity of teicoplanin against CNS has decreased over the last decade, and the use of an alternative antimicrobial may be warranted in this patient population.

P1624 In vitro activity of daptomycin and other antimicrobial agents against *Staphylococcus* coagulase-negative isolated from catheter-related bloodstream infections in intensive care unit patients

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Objectives: The present study was designed to compare the in vitro activity of daptomycin and other comparator antimicrobial agents against *Staphylococcus* coagulase-negative (CoNS) isolated from catheter related-bloodstream infections (BSI) in ICU-patients.
Methods: In vitro daptomycin activity was tested by E-test method against 64 CoNS clinical strains (*S. epidermidis* (n = 49, 76.56%), *S. hominis* (n = 11, 17.18%), *S. haemolyticus* (n = 2, 3.12%), *S. capitis* (n = 1, 1.56%), *S. warneri* (n = 1, 1.56%) collected during 2008 from 54 patients and isolated from catheter-related infections (33 from blood and 31 from the catheter). MICs were also determined for 5 comparison drugs by E-test: oxazillin, linezolid, tigecycline, vancomycin and teicoplanin and interpreted according to CLSI M100-S18 guidelines.
Results: The prevalence of methicillin resistance was (79.68%). Resistance to aminoglycosides (gentamycin), macrolides and quinolones was strongly associated with methicillin resistance (74.50%, 54.90% and 76.47% of resistance, respectively). 4.68% CoNS were non-susceptible to teicoplanin and 17.18% were non-susceptible to linezolid, but there

was no-vancomycin non-susceptibility found. The newer antibiotics, daptomycin and tigecycline, had excellent activity against CoNS.
The rank order of intrinsic activity (MIC90, mg/l) was daptomycin (1) = tigecycline (1) < vancomycin (2) < teicoplanin (4) < linezolid (32).
The most active drugs on the basis of MICs were daptomycin and tigecycline, showing MIC50 and MIC90 values of 0.25/1 and 0.125/1 mg/l and a MIC range, 0.019 to 1.5 and 0.032 to 3 mg/l, respectively. Vancomycin, teicoplanin and linezolid showed a MIC50 and MIC90 values of 1.5/2, 1.5/4 and 0.5/32 mg/l and a MIC range, 0.19 to 3, 0.125 to 24 and 0.019 to >256 mg/l, respectively.
Conclusion: Daptomycin showed an excellent in vitro activity against CoNS clinical strains isolated from catheter related-BSI, irrespective of their resistance to methicillin, aminoglycosides, macrolides and quinolones, making it a useful addition to the therapeutic armamentum for the treatment of Gram-positive catheter related-BSI.
Susceptibility testing of new compounds, as daptomycin and tigecycline, is required to establish those settings where these compounds offer real advantages over established antibiotics as glycopeptides.

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 (GPB) circulating in several European countries (Daptomycin Susceptibility of Contemporary Gram-positive pathogens circulating in Europe between 2004 and 2007. Morrissey et al. Abstract submitted to ECCMID 2009). The objective of this study was to determine the DAP susceptibility of selected Gram positive pathogens circulating in 61 centres in 9 additional European countries.
Methods: 2,305 GPB: *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 DAP MIC generally only one dilution above the break point for susceptibility. Linezolid, vancomycin and tigecylcine were also very 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 GPB 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.

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 (GPB) 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 GPB [*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 GPB 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 GPB in Europe. There was no evidence for an increase in DAP or VAN NS in currently circulating GPB 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 GPB.

P1627 In vitro activity of daptomycin combined with other antimicrobial agents against Gram-positive cocci

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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), ceftazidime (CAZ), ceftriaxone (CRO), meropenem (MEM), imipenem (IMP), ciprofloxacin (CIP), moxifloxacin (MOX), doxycycline (DOX), or 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* (EFS) and *E. faecium* (EFM) isolates (in total 50 drug combination tests with each species). The fractional inhibitory concentration indices (FICIs) were calculated to interpret the results. Synergism was defined as FICI ⩽ 0.5, indifference as FICI >0.5 to ⩽4, and antagonism as FICI > 4.
Results: Of 140 drug combination tests performed with the 10 SA strains, 125 showed no significant effect. In contrast, synergism 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, 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.

P1628 Royal jelly's effect on glucosyltransferase expression in *Streptococcus mutans*

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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. Ether 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 $60\,m \times 0.25\,mm$ (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 ($p \leqslant 0.05$) 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* ($p \leqslant 0.05$).
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.

P1629 Antimicrobial resistance of *Staphylococcus aureus* in a university hospital, Setif, Algeria, from 2002–2006

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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), kanamicin (KA), amikacine (AK), erythromycin (E), pristinamicin (PRI), ofloxacin (OFX), fucidic acid (FA), fosfomicin (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 bacteraemic 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.

P1630 *Staphylococcus aureus* from blood stream infections

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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 septicaemias 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).

Antimicrobial compound	%R	n tested	(% tested of total *S. aureus*)
Penicillin	91.57	344	(86.65)
Ampicillin/amoxicillin	91.98	262	(65.99)
Amoxicillin-clavulanic acid	28.85	104	(26.20)
Cefalothin (group)	35.71	70	(17.63)
Cefazolin	25.42	59	(14.86)
Cefotaxim (ceftriaxone)	34.29	70	(17.63)
Cefoxitin	32.43	111	(27.96)
Cefuroxime	37.14	70	(17.63)
Oxacillin	37.08	391	(98.49)
Imipenem	27.36	106	(26.70)
Erythromycin	38.66	357	(89.92)
Azithromycin	30.00	70	(17.63)
Clarithromycin	22.73	22	(5.54)
Clindamycin	28.97	390	(98.24)
Tetracycline	15.00	160	(40.30)
Doxycycline	2.19	183	(46.10)
Minocycline	0.00	52	(13.10)
Trimethroprim-sulphonamides	1.23	324	(81.61)
Norfloxacin	33.91	115	(28.97)
Ciprofloxacin	47.11	225	(56.68)
Ofloxacin	27.55	98	(24.69)
Levofloxacin	34.97	163	(41.06)
Moxifloxacin	36.18	152	(38.29)
Gentamicin	1.33	376	(94.71)
Kanamycin	13.95	43	(10.83)
Tobramycin	12.50	144	(36.27)
Nitrofurantoin	16.91	136	(34.26)
Fosfomycin	9.57	94	(23.68)
Fusidic acid	7.42	283	(71.28)
Mupirocin	1.77	113	(28.46)
Novobiocin	0.00	39	(9.82)
Rifampicin	2.10	238	(59.95)
Teicoplanin	0.00	231	(58.19)
Vancomycin	0.00	379	(95.47)
Quinupristin-Dalfopristin	2.13	94	(23.68)
Linezolid	0.00	225	(56.68)

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 septicaemic patients.

P1631 Comparison of the in vitro activity of linezolid with erythromycin against coagulase-negative staphylococci isolates

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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-replica 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 $\leqslant$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-susceptible strains are also susceptible to erythromycin.

P1632 Reversal of oxacillin resistance in MRSA with phenothiazines

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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 theses 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 phenothizines at their 1/2 MIC50 and oxacillin in the range of concentration $\leqslant$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 phenotiazines at MIC 50 ranging from 12–256 μg/ml with order of thioridizine > fluphenazine > chlorpromazine > trifluoprazine > prometazine. 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 fluphenazine 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.

P1633 Comparative susceptibility of European Gram-positive pathogens to ceftobiprole, vancomycin, teicoplanin and linezolid

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Objectives: Ceftobiprole (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 Ceftobiprole Local Antibiotic Susceptibility Surveillance Study (CLASS) compares the susceptibility of common pathogens causing serious infections in hospitalised patients. Here we report the comparative activity of BPR, vancomycin (VAN), teicoplanin (TEI) and linezolid (LIN) to Gram-positive pathogens (GPP).
Methods: 1,688 GPPs (including 281 MRSA, 302 MSSA, 250 methicillin-resistant CoNS [MRC], 174 methicillin-susceptible CoNS [MSC], 198 *Enterococcus* spp. [ES], 103 Viridans Group streptococci [VGS], 38 PRSP and 338 β-haemolytic streptrococci [BHS]) were collected from 32 centres in Austria, Germany, Ireland, Poland, Switzerland and UK during 2008. MICs were determined at each centre using Etest methodology.
Results: Against all isolates combined, BPR had the lowest MIC50 at 0.25 mg/L compared with 1 mg/L for both TEI and LIN, and 2 mg/L for VAN. BPR and LIN were equal in activity in terms of MIC90 (2 mg/L), both being lower than VAN and TEI (4 mg/L). Against MRSA, BPR had similar activity to VAN, TEI and LIN (all MIC90 of 2 mg/L). However, against MSSA, BPR MIC90 was 0.5 mg/L compared with 2 mg/L for the other three agents. Against CoNS, BPR was slightly less active than LIN against MRC (MIC90 = 2 and 1 mg/L respectively) but was similar against MSC (MIC90 = 1 for both agents). Both VAN and TEI were less active than both BPR and LIN against both groups of CoNS. Against ES, BPR and TEI had an MIC50 of 0.25 mg/L compared with 2 mg/L for both VAN and LIN but the MIC90 against ES for TEI was 1 mg/L compared with 2 mg/L for LIN and 4 mg/L for both BPR and VAN. BPR had the lowest MIC90 of the four agents against both VGS and BHS being at least 4 fold more active than VAN and LIN, and twice as active as TEI. Against PRSP, BPR had similar activity to TEI, both of which were more active than VAN and LIN.
Conclusion: BPR was highly active against a range of GPPs. Of note was that all staphylococci, regardless of their susceptibility to methicillin, were susceptible to CBP.

P1634 Activity profile of empiric respiratory tract infection agents against *Streptococcus pneumoniae* in Europe based on patient population

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Objective: The activity of antibiotics can vary based on patient location, as hospitalised patients are more likely to be infected by resistant (R) or multi-drug resistant (MDR) bacteria. For respiratory tract infection (RTI), patient location and disease severity are important criteria during the selection of empiric therapy. This study profiles empiric RTI agents against *S. pneumoniae* (SP) collected in Europe (EU) to determine R trends across varied patient populations.
Methods: During the GLOBAL 2007 surveillance program, 257 intensive-care unit (ICU), 684 inpatient (IP), 168 long-term care (LTC), and 623 outpatient (OP) isolates of SP were collected from 6 countries across EU, and were centrally tested by broth microdilution (CLSI M7-A7). Results were interpreted according to CLSI M100-S18 excluding ciprofloxacin (CIP) where FDA breakpoints were applied. MDR was

defined as R to ⩾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 >98% across PL. Similar trends were noted for CIP, though isolates were less S to CIP (91%S) than LVX (99%S) overall. MIC90 s of the β-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 β-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 >97%S to LVX and LVX remained the most potent agent tested against SP. Variation in S to β-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.

P1635 Activity of zabofloxacin, a new fluoroquinolone, against common respiratory pathogens, compared with five other fluoroquinolones

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Objectives: Zabofloxacin (DW-224a, PB-101), a new fluoroquinolone, has broad in vitro antibacterial activity against Gram-negative and Gram-positive organisms, including levofloxacin (Levo) resistant *S. pneumoniae* (QRSP). This study compared the in vitro activity of Zabo and 5 other quinolone antibiotics against clinical isolates of *H. influenzae* (H.flu), and *S. pneumoniae* (S.pn), including levofloxacin resistant strains.
Methods: MICs (CLSI methodology) were determined by agar dilution method against clinical isolates of H.flu (233) and S.pn (264) including 24 levofloxacin resistant strains collected from 1997 to 2008 in South Korea.
Results: The MIC50/MIC90 of Zabo against H.flu are similar those of other quinolones 0.016/0.03 ug/ml. Against S.pn, the MIC90 of Zabo was 0.03 ug/ml and 0.5 ug/ml against the Levo susceptible and resistant strains, respectively. MIC50 and MIC90 values for Zabo and comparators are presented in the table.

Quinolone	Levofloxacin susceptible S.pn (240)			Levofloxacin resistant S.pn (24)		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Zabofloxacin	0.08–1	0.015	0.03	0.03–0.5	0.25	0.5
Moxifloxacin	0.06–1	0.25	0.25	1–>8	4	8
Levofloxacin	0.25–4	1	1	8–>16	16	16
Gemifloxacin	0.015–0.5	0.03	0.06	0.06–2	1	1
Gatifloxacin	0.125–4	0.5	0.5	2–>8	8	8
Ciprofloxacin	0.25–16	2	4	8–>32	>32	>32

Conclusion: Zabo was the most active of all tested quinolones against clinical isolates of S. pn including levofloxacin resistant.

P1636 Report of linezolid resistance from the Zyvox® Annual Appraisal of Potency and Spectrum Program (Europe, Latin America, Asia Pacific)

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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 β-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

Species	City/Country	LZD MIC (mg/L)	R-mechanism (23S mutation)
S. aureus	Dublin/Ireland	8	G2576T
S. epidermidis[a]	Rome/Italy	8	G2576T
S. epidermidis[a]	Rome/Italy	8	G2576T
E. faecalis	Brasilia/Brazil	>8	G2576T

[a]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 erythromicin resistance rates of 20.1% and 33.6%, respectively. All streptococci had LZD MIC values of ⩽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

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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 (⩽0.25/0.5/1/2 mg/L).

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; M7-A7) 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 (0.06/0.12 US; 0.06/0.25 EU). Against SA with VAN MICs of 0.5 and 1 mg/L, ORI maintained potent activity with MIC50 s from 0.03–0.06 mg/L and MIC90 s 0.06–0.25 mg/L among evaluated SURV isolates from 2005–2008. However, ORI MIC50 s and MIC90 s against SA with VAN MICs of 2 mg/L (MIC50 s of 0.12–0.25 mg/L; MIC90 s of 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 MIC50 s and MIC90 s 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.

P1638 Update on daptomycin activity and spectrum when tested against Gram-positive strains collected in European medical centres (2007–2008)

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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 mainly from bloodstream infections (49%) and cSSSI (20%). The strains were susceptibility (S) tested against DAP and numerous comparators by CLSI broth microdilution methods in cation-adjusted Mueller-Hinton broth supplemented to 50 mg/L of calcium for DAP tests.
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).

Organism (no. tested)	Cumulative % inhibited at daptomycin MIC (mg/L) of:						%S
	⩽0.12	0.25	0.5	1	2	4	
SA							
OXA-S (4,047)	5.7	86.5	99.7	100.0	–	–	100.0
OXA-R (1,531)	4.0	76.5	99.1	100.0	–	–	100.0
CoNS (1,665)	10.2	65.3	96.0	99.9	100.0	–	99.9
EF (1,306)	0.5	3.2	37.3	92.2	99.9	100.0	100.0
EFM							
VAN-S (533)	0.9	2.1	7.1	32.7	94.0	100.0	100.0
VAN-R (210)	0.0	1.4	4.3	42.9	96.2	100.0	100.0
BHS (807)	83.9	99.4	100.0	–	–	–	100.0
VGS (274)	31.4	65.7	92.3	100.0	–	–	100.0

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% S) 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)

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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 (cSSTI) 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, 128) hospitals in the 2006–2008. MIC was assessed by reference CLSI broth microdilution method in cation-adjusted Mueller-Hinton broth, additionally supplemented to 50 mg/L of calcium for daptomycin testing. VAN MBC and the presence of VAN heteroresistant population (hVISA) were evaluated in a randomly selected subset of 31 isolates. MBC was assessed by plating the entire volume of the broth from each well above the MIC for each organism. The lowest concentration of antimicrobial agent that killed ⩾99.9% of the initial inoculum was defined as the MBC endpoint. hVISA was evaluated using the GRD Etest strip (AB Biodisk, Solna Sweden) according to manufacturer's instructions.
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 daptomycin MIC 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 MBC was ⩾32 mg/L in 12.9% of strains and 25.8% of strains tested for hVISA were positive.

Table. MRSA with VAN MICs at 2 mg/L

Region (no of isolates)	Cumulative % inhibited at daptomycin MIC (mg/L) of:					MIC_{50}	MIC_{90}
	0.25	0.5	1	2	4		
Europe (128)	31.3	93.0	100.0	–	–	0.5	0.5
United States (282)	29.4	89.4	96.1	98.9	100.0	0.5	1

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.

P1640 Susceptibility trends for methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* bloodstream isolates over a 2.5 year period

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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 (bioMerieux, 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.

Table 1

Strain (Range, MIC or MBC μg/ml, or MBC/MIC ratio)	Method	Correlation coefficient, P-value
MRSA (0.19–3)	D Etest	−0.307, $p \leqslant 0.001$
MRSA (1–4)	V Etest	−0.178, $p \leqslant 0.001$
MRSA (0.5–2)	L Etest	−0.382, $p \leqslant 0.001$
MRSA (MBC range 0.25–64)	MBC V	−0.186, $p \leqslant 0.001$
MRSA (MBC/MIC range 1–128)	MBC/MIC ratio V	−0.221, $p \leqslant 0.001$
MSSA (0.25–1)	Manual MIC V	+0.250, $p = 0.014$
MSSA (0.12–1)	D Etest	−0.227, $p = 0.026$
MSSA (MBC range 0.25–64)	MBC V	+0.251, $p = 0.013$
MRSA USA100/USA 300 (0.19–2/0.25–2)	D Etest	−0.267, $p \leqslant 0.001$ / −0.412, $p \leqslant 0.001$
MRSA USA100/USA 300 (1–4/1–2)	V Etest	−0.209, $p = 0.006$ / −0.182, $p = 0.028$
MRSA USA100/USA 300 (0.75–2/0.5–2)	L Etest	−0.426, $p \leqslant 0.001$ / −0.311, $p \leqslant 0.001$
MRSA USA100/USA 300 (MBC range 0.25–64/0.25–64)	MBC V	−0.262, $p \leqslant 0.001$ / −0.168, $p = 0.042$
MRSA USA100/USA 300 (MBC/MIC range 1–128/1–128)	MBC/MIC ratio V	−0.250, $p = 0.001$ / −0.192, $p = 0.020$

For MRSA, and specifically both USA100 and USA300 strains, a significant decrease across time was detected in MIC's for D, V and L using Etests, as well as MBC's and tolerance for V. A significant decrease with the MSSA was seen in the D Etest, while a significant increase was noted across 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 MRSA 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.

P1641 Antibiotic susceptibility patterns of *Staphylococcus aureus* in north-eastern Romania and comparison of phenotypic methods for detection of oxacillin resistance

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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 (bioMerieux, 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 (bioMerieux, France), oxacillin E-test strips (AB Biodisk, Sweden) and a penicillin-binding protein (PBP) 2a latex agglutination test (Slidex MRSA, bioMerieux, 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 cotrimoxazol). MSSA have low resistance rates to other antibiotics. All strains were susceptible to vancomycin, 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.

P1642 In vitro activity of daptomycin against staphylococcal and streptococcal isolates from orthopaedic patients

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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: A total of 110 non-duplicate clinical isolates were selected, including 35 methicillin-resistant *Staphylococcus aureus* (MRSA), 30 methicillin-susceptible *S. aureus* (MSSA), 30 methicillin-resistant *Staphylococcus epidermidis* (MSSE), and 15 group A Streptococci (*S. pyogenes*). MICs of penicillin, oxacillin, erythromycin, clindamycin, gentamicin, fucidic acid, ciprofloxacin, moxifloxacin, 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.
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, fucidic 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 daptomycin 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 daptomycin, which could be a new therapeutic option in the treatment of infections in orthopaedic patients.

Clean(ing) hospitals

P1643 Does targeted and quantified control of the microbiological environment within the intensive care unit reduce colonisation and healthcare-acquired infection?

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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

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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

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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 sporicidal. However, it poses health and safety concerns and may damage the hospital environment. The AzoMaxActive™ range of cleaning products contain Byotrol™ technology, a novel combination of biocides sequestered to a nanopolymer backbone, which is sporicidal 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 Brazier'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 > 100 or >10 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 numbers 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.

Sites +ve for	Test	Control	*p*
TVC > 10	1211 (58.2%)	1575 (75.7%)	<0.0001
TVC > 100	436 (21.0%)	627 (30.1%)	<0.0001
MRSA Direct	31 (1.5%)	37 (1.8%)	ns
MRSA Total	45 (2.2%)	46 (2.2%)	ns
C. difficile	12 (0.6%)	8 (0.4%)	ns

P1646 High sporocidal activity using dissolved chlorine dioxide (SanDes™) on different surface materials contaminated by *Clostridium difficile* spores

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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 48 h on FAA and CFU count was recorded.
Results: The mean concentration of inoculum was 25×10^9 cells/L ($12–31\times10^9$/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, bedrails (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*

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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 lateral 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/mm^3 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 1. Culture results of the disks according to variables

	Growth n* (%)	No growth n* (%)	Total n* (%)	p
Disks in				
opened plates	4 (8.3)	44 (91.7)	48 (100)	<0.001
closed plates	9 (56.3)	7 (43.8)	16 (100)	
Disks				
outside the drawer	2 (4.2)	46 (95.8)	48 (100)	<0.001
inside the drawer	11 (68.8)	5 (31.3)	16 (100)	
Disks containing				
pure suspension	4 (12.5)	28 (87.5)	32 (100)	>0.05
serum + suspension	9 (28.1)	23 (71.9)	32 (100)	

*n = The number of disks.

P1648 Efficacy of selected weak organic acids against multi-antibiotic-resistant planktonic and biofilm bacteria

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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

1%w/v NaCl. Microtiter plates were inoculated with bacterial suspension to establish biofilms.
Biofilm formation was detected by staining wells with crystal violet and then resolve the crystal violet in a mixture (2:8) of acetone and ethanol. Biofilm formation was confirmed, if the reading at 570 nm of the positive control was at least three times more than the reading of the negative control. Wells were exposed to WOAs in different concentration, washed with distilled water and RPMI-1640 growth media was added detecting any bacterial growth at 570 nm.
Results: For planktonic Gram-positive bacteria (MRSA, VRE) at least 8%w/v concentrations of WOAs and their combinations were effective after 6 to 10 minutes exposure. For planktonic Gram-negative bacteria (MRAB, ESBL positive *Klebsiella pneumoniae*) at least 2.5%w/v concentrations of WOAs and their combinations were effective after 2 minutes exposure. Lactic acid alone and in combination with malic acid showed to be the most effective against both type of bacteria. Individual and combination of WOAs in 10%w/v concentration after 60 minutes exposure were capable to penetrate into biofilms and eliminate bacteria.
Conclusion: Our results indicate that WOAs, especially lactic acid and combinations thereof, are effective bactericidal agents against planktonic Gram-negative and Gram-positive multiresistant bacteria. Enhanced efficacy with Gram-negative bacteria is probably due to their lipopolysaccharide layer, which is more permeable to acids due to the acid soluble lipid component. These WOAs alone or in combination are also capable to penetrate into biofilms, increasing their potential to be used as sanitizer.

P1649 Efficacy of selected weak organic acids against enveloped and non-enveloped viruses and an intracellular bacterium

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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 bacteria.
Four organic acids, ascorbic, 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. Uninfected 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.

Pharmacokinetics and pharmacodynamics of antibacterials and antifungals

P1650 Target controlled infusion as a mode of i.v. administration for time-dependent antibiotics

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Objective: In many countries, approved Target controlled infusion (TCI) pumps are widely used for anaesthetic and analgesic drugs. These devices could be easily adapted for selected antibiotics by the incorporation of an appropriate pharmacokinetic (PK) model such that the system will aim to achieve a desired target drug concentration in blood and tissues. The aim of this ongoing study is to identify suitable candidates to test the hypothesis that the TCI mode of administration could provide clinical advantages.
Methods: Advantages of TCI over conventional continuous infusion are (i) improved individualisation of dosage if a well characterised population PK model is available (ii) target concentration profiles can be determined to achieve effective drug concentrations in tissues more quickly and (iii) proportional changes in the target concentration can be made if drug monitoring reveals inadequate or excessive concentrations. Computer simulation is being used to examine the PK of those antibiotic agents that have been advocated for use by continuous infusion. Cumulative drug delivery over time is used to compare the influence of different published PK models for the same drug. Different target concentration profiles are being explored to determine the rate of equilibration between predicted blood and tissue concentrations.
Results: With vancomycin as an example, cumulative drug delivery over 24 hr for a 70 kg patient, with a blood target concentration (CbT) of 15 mcg.ml^{1}, ranged from 1803 to 2878 mg with 7 different PK models. With CbT of 20 mcg.ml^{1} for 1 hr followed by 10 mcg.ml^{1} and a population PK model described by Mulla (2005), 24 hr drug delivery was 1415 mg in an adult patient with serum creatinine (Cr) 80 mmol.l^{1}, 940 mg in an adult with Cr 160 mmol.l^{1} and 385 mg in a 5 yr, 18 kg child with Cr 80 mmol.l^{1}. This latter CbT profile achieves equilibration between predicted blood and tissue vancomycin concentrations of 10 mcg.ml^{1} within 2 h and predicted concentrations remain stable thereafter.
Conclusions: In line with the plea that currently available drugs should be administered in the optimum fashion, TCI may provide a new option worthy of investigation. Once potential candidates are identified, it will be desirable to achieve a consensus among PK experts on the most appropriate PK models for particular drugs and populations.

P1651 New horizons to modern therapeutic drug monitoring – use of tandem mass spectrometry to analyze the 40 most important anti-infectives

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Objectives: Previously only very few anti-infectives could be measured within a reasonable time to allow a physician to react to reported drug levels. This is particularly regrettable since pharmacodynamic principles have begun to be ready for clinical use, yet needing validation in the "real world". Modern tandem mass spectrometry has opened a new era to measure anti-infectives with relatively short development time for the assay and quick response to urgent needs for blood or tissue levels from the clinic.
Methods: We developed tandem mass spectrometry for the most important forty anti-infectives (antibiotics, anti-TB-agents, antivirals, anti-fungals, anti-malarials), applied the strict FDA-Guidelines for Bioanalytical Work and set up a quality assurance system. Precursor → product ion transitions for representative anti-infectives such as: 548 → 539 caspofungin, 547 → 468 ceftazidime, 332 → 314 ciprofloxacin, 198 → 108 clavulanic acid, 811 → 159 daptomycin, 474 → 265 ertapenem, 735 → 158 erythromycin, 307 → 238 fluconazole,

137 → 79 fosfomycin, 300 → 98 imipenem, 338 → 296 linezolid, 384 → 68 meropenem, 172 → 128 metronidazole, 516 → 330 piperacillin, 245 → 113 ribavirin, 232 → 140 sulbactam, 254 → 156 sulfamethoxazole, 299 → 138 tazobactam, 586 → 569 tigecycline, 291 → 230 trimethoprim, 725 → 144 vancomycin, 350 → 127 voriconazole.
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

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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 acetonitrile.
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

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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 Fcgamma- and 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 Fcgamma 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) Fcgamma 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?

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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 physicochemical properties of the agents.
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 ceftidoren. That should allow a long awaited valid comparison between agents.
Results: ELF to plasma ratio: Trovafloxacin: 2.75±1.933, Ceftidoren: 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

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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.

P1656 Effects of azidothymidine on apoptotic cell death: role of nuclear factor kB

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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 monocytic tumour 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 a 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-mIKB 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.

P1657 Post-antibiotic effect of various antibiotics on *Legionella pneumophila* strains isolated from water systems

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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.

P1658 Combination therapy for multidrug-resistant *Acinetobacter baumannii* in a neutropenic murine pneumonia model

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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 10^7 CFU of MDR AB intratracheally under laryngoscopic guidance. Serum TNF-α and IL-6 were measured 24 hours 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 ($p < 0.01$). 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.

P1659 Testing the antibacterial and antifungic activity of the species *Pelargonium roseum* and *Pelargonium graveolens*

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Objectives: The objective of the present study is to test the antibacterial and antifungic activity of essential oils yielded by *Pelargonium roseum* and *Pelargonium graveolens*.

Methods: In order to test their antibacterial and antifungic activity, we made use of the radial diffusimetric method, impregnating round filtre disks made of paper with essential oil yielded by the above-mentioned plants.

Previously sterilised 6 mm diameter round disks were used. Onto each we dripped 0.01 ml essential oil, using a sterile semi-automatic micropipette. The paper disks were put in Petri plates, onto simple gelose cultures, which were preserved at 37ºC in thermostat during 24 h. Reading was done by measuring the diametres of inhibation of microbial development around the round disks.

The microorganisms used for testing the antimicrobial activity of essential oils were selected from the standardised microorganism collection of Tg-Mures Public Health Centre. We used the following bacterial stems:

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 (cyprofloxacine, cotrimoxazole, gentamycine, amykacin, cephotaxim, cephalotin).

Results: The results obtained are presented in the table.

Stem being tested	Inhibition zone (mm)													
	Essential oils							Antibiotics						
								ciprofloxacin	ampicillin	cotrimoxazole	gentamicin	amikacin	cephotaxim	cephalotin
Code	1263	1264	1265	1266	1267	1268	1269							
Sample	1	2	4	5	6	7	8							
Pseudomonas aeruginosa ATCC 27853	22	26	18	20	26	20	31	30	11	11	19	21	24	10
Proteus mirabilis ATCC 29906	11	13	12	10	17	16	13	35	35	28	20	20	35	38
Staphylococcus aureus ATCC 25923	21	28	22	21	25	25	31	25	29	28	22	19	35	38
Escherichia coli ATCC 25922	14	15	10	11	11	11	15	28	23	25	17	16	36	16
Enterococcus faecalis ATCC 19433	22	15	16	12	10	15	13	22	30	25	17	16	37	26
Candida albicans ATCC 10231	compl	compl	compl	compl	compl	compl	compl	–	–	–	–	–	–	–

Sample 1 – essential oil of *Pelargonium graveolens* (China)
Sample 2 – essential oil of *Pelargonium roseum* (Reunion)
Sample 4 – essential oil of *Pelargonium roseum* (Targu-Mures, 2004)
Sample 5 – essential oil of *Pelargonium roseum* (France)
Sample 6 – essential oil of *Pelargonium roseum* (Targu-Mures, 2005)
Sample 7 – essential oil of *Pelargonium graveolens* (Egypt)
Sample 8 – essential oil of *Pelargonium roseum* (Targu-Mures, 2003)

Conclusions: In case of *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) an inhibition comparable with that of microcapsules containing antibiotics was discovered. No significant differences between the two types of essential oils being studied in the present study were found. As to their bioactivity against *Candida albicans*, a complete inhibition of the development of this fungus was found.

Further studies, made on the basis of the dilution method, are needed in order to determine the minimal concentration of the inhibitory effect of geranium (*Pelargonium*) essential oils against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and respectively, *Candida albicans*.

P1660 Pharmacokinetics of colistin after intravenous administration in critically ill patients receiving continuous venovenous haemodiafiltration

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Objectives: Colistin is used in infections by multi-drug resistant Gram-negative bacteria (MDR-GNB) but pharmacokinetic data are limited, especially in patients receiving continuous renal replacement therapy (CRRT). The pharmacokinetics of colistimethate sodium (CMS) and colistin in a population of critically ill receiving CRRT was studied.

Methods: Critically ill patients on renal replacement therapy through continuous venovenous haemodiafiltration (CVVHDF) receiving colistin for infection by MDR-GNB were enrolled. CVVHDF was performed using an AN 69 HF hollow fibre haemofilter/dialyser (Prisma M100 Pre Set; Hospal Industrie, Meyzieu, France)with an effective surface area of 0.90 m2. CMS was administered at a dose of 2 MU (160 mg) q8 h. Venous, afferent and efferent blood was collected immediately before and at multiple occasions after the fourth infusion. Plasma CMS and colistin concentrations were determined with a novel LC-MS/MS method after a rapid precipitation step that avoids significant degradation of CMS and colistin.

Results: Five patients (3 female, mean age 65.8 years) were included. Mean (SD) blood flow rate, substitution and dialysate flow rates were 134 (15.2) ml/min, 1120 (779.9) ml/h and 1430 (383.4) ml/h respectively. Selected pharmacokinetic parameters are shown in Table 1.

Table 1. Calculated PK parameters, mean (SD).

	CMS	Colistin
C_{max} (mg/L)	4.93 (2.01)	0.92 (0.46)
C_{min} (mg/L)	1.08 (0.40)	–
$T_{1/2}$ (terminal, h)	3.29 (0.62)	–
Extraction (%)	30 (13)	68 (8)
CL_{HDF} (L/h)	2.45 (1.26)	5.5 (1.10)

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.

P1661 Pharmacodynamic efficacy of levofloxacin against multidrug-resistant *Pseudomonas aeruginosa* isolated from patients with ventilator-associated pneumonia

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Objectives: Although levofloxacin (LVF) has been proposed for the treatment of ventilator-associated pneumonia (Benko R, et al. IJAA 2007; 30: 162), there is a lot of scepticism about the appropriateness of its administration in settings with high-resistance rates. The in vitro effect of LVF at concentrations close to those achieved in the epithelial lining fluid (ELF) against multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) pathogens from patients with ventilator-associated pneumonia (VAP) was assessed.

Methods: Eleven MDRPA from patients with VAP enrolled in a prospective trial (NCT00297674) were tested. Pathogens were grown at a density greater than 1×10^6/ml from tracheobronchial secretions in parallel with a clinical pulmonary infection score more than 6. A log-phase inoculum of 1×10^6 cfu/ml of pathogens was exposed over time to 5, 10 and 25 microg/ml of LVF that corresponds to its ELF levels. They were also exposed to 18 microg/ml of meropenem (MER), to 5 microg/ml of colistin (COL) and to their interactions. The latter are

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 bactericidal 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, five 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 MDRPA pathogens of VAP when applied at concentrations close to ELF. Single MER and COL were not that effective but they acted in synergy with LFV. These findings reinforce the application of LVF for VAP by MDRPA.

P1662 New insights into the most commonly studied drug interaction with antibiotics: pharmacokinetic interaction between ciprofloxacin, gemifloxacin and probenecid at renal and non-renal sites

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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 between CIP and gemifloxacin. 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%] ($p < 0.08$) 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.

P1663 Absorption, distribution, metabolism and excretion of zabofloxacin (DW-224a, PB-101) after a single oral administration

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Objectives: To examine the pharmacokinetics, tissue distribution, metabolism and excretion of Zabofloxacin (also known as DW-224a and PB-101), a new broad-spectrum fluoroquinolone-type antibiotic with enhanced in vitro activity against *Streptococcus pneumoniae*.
Methods: [14C]Zabofloxacin hydrochloride (100 mg salt/kg), as a solution in purified water, was dosed to fasted male Sprague-Dawley rats and male Lister-Hooded rats.
Results: After oral administration of [14C]Zabofloxacin 93.9% of radiolabelled dose was recovered after seven days of which 79% dose was eliminated in faeces and about 13% dose excreted in urine. In bile duct-cannulated rats, 84% of the dose was recovered in faeces with 3.7% and 3.4% dose excreted in urine and bile, respectively. The total circulating drug-related material increased to a Cmax of 5.39 μg equivalents free base/mL after 1 h, and then declined (to 1% of Cmax at 12 h post-dose). Tissue concentrations of drug-related material were generally maximal between 0.5–1 h post-dose, with a later Cmax observed in gastrointestinal tract, testes and skin. In pigmented rats, tissue concentrations were highest in the eyes and remained measurable over 112-days. Essentially, all radioactivity excreted in faeces and urine was unchanged Zabofloxacin, as was the majority within the kidney and liver. In bile, Zabofloxacin was essentially absent but up to three major metabolites were present. In circulating plasma, whilst Zabofloxacin was detectable, a conjugate of Zabofloxacin was also prominent, and this Phase II metabolite was also detectable in a number of tissue matrices (lung, testes, thymus).
Conclusion: [14C]Zabofloxacin hydrochloride orally administered demonstrated good absorption and generally higher tissue concentrations than plasma levels. The majority of dose was recovered in the faeces followed by the urine and bile. Drug-related material was found to bind to pigmented tissue particular ocular melanin. Minimal metabolism was found in the faeces and urine but up to three major metabolites were detected in bile with conjugates present in plasma and tissues.

P1664 Toxicodynamics of itraconazole

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Objective: To identify the frequency of adverse events that occurred during treatment with itraconazole (ITZ), and examine the relationship between ITZ concentrations and the probability of toxicity.
Methods: Patients who received ITZ and underwent therapeutic drug monitoring (TDM) were identified from the Regional Mycology Laboratory database. The following information was obtained from patients' medical records: age, gender, diagnosis, ITZ dosages and duration of treatment. All plasma ITZ concentrations were measured by bioassay. Toxicity was defined as any symptom, sign or biochemical disturbance that occurred during treatment and resolved or improved with ITZ withdrawal or dose-reduction. Observed features of toxicity were sub-classified as gastro-intestinal (GI), cutaneous, metabolic disturbance, and symptoms of fluid retention. For patients with toxicity, the mean ITZ concentration in the 3 months preceding an adverse event were calculated. These were compared to ITZ concentrations in an equivalent 3-month-period for patients without adverse events. The relationship between mean ITZ concentrations and the probability of an adverse event was defined using logistic regression.
Results: Data from 216 patients were available for analysis. The overall rate of toxicity was 47%. The incidence of GI symptoms, fluid retention, cutaneous symptoms, and metabolic disturbance were 21, 18, 7 and 4%, respectively. The mean±SD ITZ concentrations in patients experiencing an adverse event versus patients not experiencing an adverse event were 7.0±5.9 and 16.0±8.7 mg/L, respectively ($p < 0.001$). Logistic regression demonstrated a progressive increase in the probability of an adverse

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

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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.

Subjects/ parameters	Cmax (µg/mL)	T_{max} (h)	$AUC_{0-\infty}$ (µg·h/mL)	CL/F (L/h)	$T_{1/2}$ (h)	V/F (L)
Healthy	0.843±0.171	1.5–3.0	44.6±11.3	2.38±0.640	148±73.2	480±149
Impairment						
Mild	0.729±0.165	1.0–4.0	97.8±52.8	1.26±0.550	292±117	485±212
Moderate	0.472±0.122	2.0–4.0	62.2±24.9	1.82±0.640	240±58.3	598±183

Arithmetic mean (±SD), except for T_{max} presented as range.

P1666 Skin concentrations and pharmacokinetics of posaconazole after oral administration

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Objectives: The primary objective 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 ng/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.

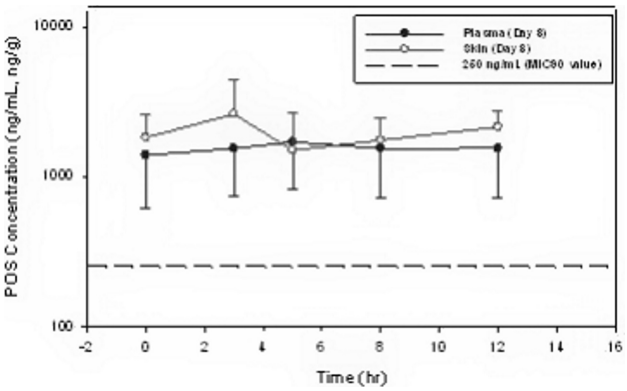

Figure 1. Mean plasma ± 1 SD) and skin concentration-time profiles of posaconazole on Day 8 after oral administration of posaconazole (400 mg BID) in healthy subjects.

Conclusions: AUC/MIC90 ratio in skin and plasma was similar. POS skin concentration on Day 8 remained several-fold above the MIC90 value[1] for dermatophytes commonly seen in cutaneous infections. These findings demonstrate adequate skin penetration and have implications for treatment of dermatophytic skin and nail infections.

Reference(s)

[1] A. K. Gupta, Y. Kohl, and R. Batra. In vitro activities of posaconazole, ravuconazole, terbinafine, itraconazole and fluconazole against dermatophyte, yeast and non-dermatophyte species. Medical Mycology March 2005, 43 (179–185).

P1667 Inhibition effects of four antifungal triazoles (itraconazole, fluconazole, voriconazole and posaconazole) on specific activities of CYP3A4 in human liver microsomes

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Triazoles 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 cytochrome 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 μM and 75 μM ketoconazole, midazolam metabolism from 5 μM 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).

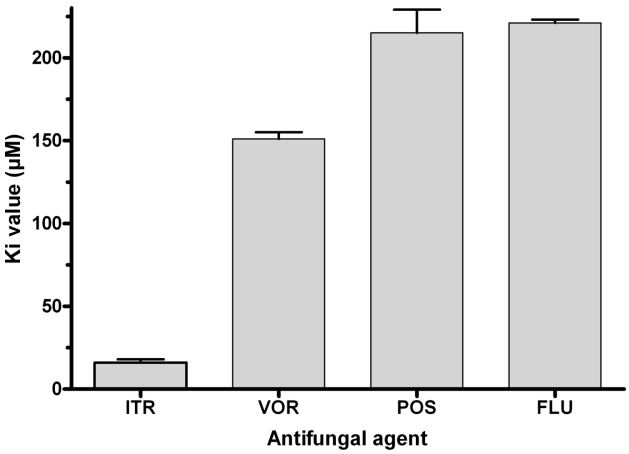

Figure 1. Ki values of antifungal triazoles against CYP3A4 activities. A higher Ki indicates a lower inhibition of CY3A4.

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.

P1668 Development and validation of a liquid chromatography-tandem mass spectrometry procedure for the quantification of tigecycline in human serum: application to drug monitoring

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Objectives: There is general agreement that following new drug approval more work needs to be done to further understand an agents clinical properties. Therapeutic drug monitoring is one way to help assess a new agent in a broader range of patients.
For that purpose we developed and validated (according to FDA-Guideline) an assay for the determination of tigecycline in human serum.
Methods: Determination of tigecyclin in human serum 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, 596 → 579 for d9-tigecycline. The plasma samples were extracted with organic solvent including internal standard and injected into the LC-MS/MS System. Chromatography was performed on a reversed-phase analytical column with a mobile phase consisting of ammonium formate and acetonitrile.
Results: Linearity could be shown within 0.0100–10.0 μg/mL and correlation coefficients were at least 0.998 on all validation days. Intra-run CVs ranged from 0.5 to 8.3%, inter-run precision (CV) ranged from 1.6 to 4.8%. Mean recovery over the whole concentration range was 90.2%±4.2% SD for tigecycline and 90.5%±2.3% SD for d9-tigecycline. There was no instability observed for tigecycline during the validation procedure. Prepared samples are stable for at least 48 hrs at +4ºC. Serum samples are stable for at least 5 months at −70ºC and up to three freeze and thaw cycles. Haemolytic, lipaemic and six different plasmas showed no effect on the determination of tigecycline. Also diluting samples has no influence on the measurement. Blinded sample analysis precision ranged from 0.7 to 10.3%. Cross validation CVs to heparine, EDTA and citrate plasma ranged from 0.8% to 11.3%.
Conclusion: We developed and validated a method for the determination of tigecycline in human serum and plasma. Due to short analytical runtime we can analyze over 300 samples per day per LC-MS/MS system according to GLP guidelines. Analysing clinical drug monitoring serum and plasma samples demonstrated the excellent performance of the assay.

P1669 Effect of protein binding in the activity of voriconazole and anidulafungin alone or combined against *Aspergillus* sp. using a time-kill methodology

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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 MIC ANF= 0.03 and 0.12 mg/L) strains.
Methods: Killing curves were performed with a final inoculum of approximately 10^5 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 58%, and 99% protein binding for VOR for ANF, respectively. Control growth curves were performed in all media tested without antibiotics. Cultures were incubated at 35ºC. 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.

Strain/MEC (mg/L)	Cmax	Cmax-S	Cmax-Alb	CmaxF
A. fumigatus 5/0.015	42.44	50.55	45.57	100.00
A. fumigatus 8/0.12	40.00	35.00	70.49	100.00
A. flavus 9/0.12	100.00	64.91	100.00	98.28
A. flavus 10/0.03	87.06	59.8	77.32	92.78

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 collaborations, provides information on funding opportunities, organises training

events, and acts as a forum for encouraging high quality translational infectious disease research.
Methods: We achieve this by several methods. These include running of research strategy workshops to identify priority research topics and providing support to groups who are focussed on a specific research question. Support may include arranging further meetings, identifying extra collaborators and assisting with the financial projections for the project. We also run training events, and have various electronic resources, such as current funding and training bulletins, and an online researchers database.
Results: IDRN research outcomes include Flu-Watch (household cohort study on transmission of influenza), National Intestinal Infectious Disease Study (looking at diarrhoeal illness in the community) and a Medical Research Council fellowship looking at community-acquired MRSA. See the 'research' page on the IDRN website for a complete list.
Conclusions: The IDRN provides a model of how a small administrative infrastructure, with academic input from Network members, can initiate and support a wide range of collaborations, training events and information resources, ultimately leading to better collaborative research. Our website is www.idrn.org

P1671 Biological risk in a pharmaceutical dump: *Brucella abortus* strain viable after 20 years of disposal

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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 analyzed. 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* (JCM, 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 antibioresistance hallmarks in *Escherichia coli* strains isolated from Black Sea water on Romanian coast

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Purpose: The aim of this study was to investigate by phenotypic and molecular techniques the antibioresistance profile and the virulence markers of 100 environmental *Escherichia coli* aquatic isolated marine water in Constanta, Romania.
Material and Methods: The antibiosusceptibility testings were performed by disk diffusion method (CLSI, 2008) and phenotypic screening of β-lactamases. The presence of different antibioresistance 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 antibioresistance and virulence genes: blaTEM-like, blaSHV-like, blaOXA-like, blaCTX-M-like.
Results: The tested strains exhibited high susceptibility to imipenem, ceftriaxone, cefoxitin and nitrofurantoin (99%), tobramycin (98%), cephtazidime (97%), gentamycin and amikacin (96%), ciprofloxacin (93%), nalidixic acid and chloramphenicole (92%) sulphametoxazole (89%) and high resistance levels to ampicillin (94%). The tested strains exhibited between 1 and 8 antibioresistance markers, the most frequent associations being: AMP+SXT (10%), AMP+C (9%), AMP+CIP (8%) and AMP+NA (7%). Morevoer, 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 nitrocephine 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 harboured 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%). Unexpectedely, 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%), lysin-decarboxilase (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

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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%. Tthere 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).

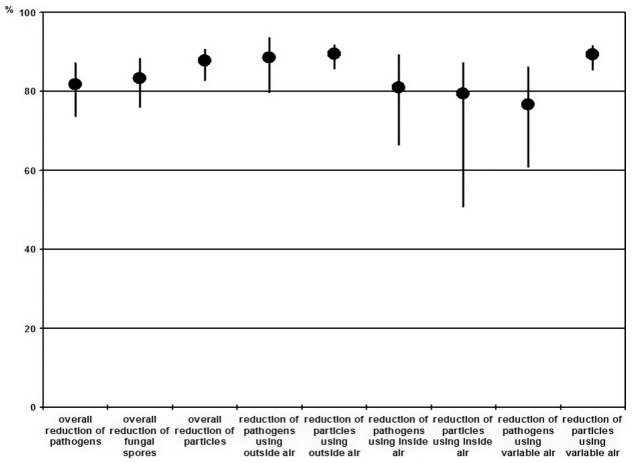

Figure 1.

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 in use. Most probably the risk for an allergic reaction will be reduced during use also.

P1674 Characterisation of bacterial isolates in cheese production surveillance in an organic farming site

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Objectives: During the last decade, organic farming became popular in Germany. Therefore, demand for surveillance in such facilities increased accordingly. In cheese production from sheep milk it is not only necessary to screen the final product, but also to screen the milk and the udders. Furthermore, transmission of pathogens from the farmers and their families to live-stock and to raw milk have to be ruled out.
Methods: At an organic farm, milk and cheese samples as well as swabs from sheep and farmers were collected. Subsequently, they were subjected to analysis using standard microbiological screening as well as molecular techniques, such as PCR, sequencing and diagnostic microarrays to characterise the bacterial isolates.
Results: By growing the different samples from cheese, milk, humans and sheep on Columbia blood agar plates and subsequent microbiological and molecular analysis, 13 different bacterial species were identified and confirmed by sequencing, PCR and diagnostic microarrays. Most of them belonged to the genus of *Staphylococcus*, representing the species *S. warneri*, *S. chromogenes*, *S. fleuretti*, *S. xylosus*, *S. equorum*, *S. epidermidis* and *S. aureus*. But also other bacteria as *Kocuria* spec., *Acinetobacter* spec., *Moraxella* spec., *Corynebacterium* spec. and *Streptococcus pyogenes* were identified. All staphylococci were analysed for 334 different sequences representing species, virulence and antimicrobial resistance markers using the StaphyType-Kit. By this method, a unique fingerprint of a *S. aureus* isolate was generated allowing to trace transmission of the agent. No MRSA or PVL positive isolate was detected. Most interestingly, the isolates from a human wound swab and from the cheese sample were completely identical. It was an agrIV/CC121, capsule type 8 isolate that contained no antimicrobial resistance genes but yielded signals for enterotoxins G, I, M, N and O and U. PVL and mecA were not present. Another isolate cultured from a human nasal swab was identified by 16S sequencing as *S. fleuretti*. It was mecA positive and additionally contained the genes mecI, mecR, ugpQ and xylR. The isolates from sheep udders (partially suffering from mastitis) were all identical. They were agrI/cc133 MSSA containing enterotoxins C and L as well as leukocidin components lukM and lukF-P83.
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

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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 menigococci 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 (Pliva Lachema) and serogrouped with latex agglutination tests: BD Directigen Meningitis (Becton Dickinson), Pastorex Meningitidis (BioRad) and PCR method. Susceptibility patterns to antibiotics: penicillin, ceftriaxone, rifampicin and ciprofloxacin were determined using E-tests. Molecular characteristic was performed by PFGE method with SpeI restriction enzyme and computer analysis with Molecular Analyst (BioRad) software application.
Results: The carriage rate of *N. meningitidis* in the region was estimated at 3.03% (41individuals). 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 *Haemophilus influenzae* lipopolysaccharides in a healthy population in Scotland?

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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

pneumonia: *Acinetobacter baumannii* and *Haemophilus influenzae*, in the healthy population. Our objective was to determine how widespread antibody levels (as indicators of exposure) were to the lipopolysaccharides (LPS) of these two species. These were compared to antibody levels to the LPSs of *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* of which we know more about antibody levels.
Methods: 475 serum samples from healthy donors were collected from the Southeast Scotland Blood Transfusion Service. These were used in ELISAs against LPS extracted by the aqueous phenol method from 8 strains of *A. baumannii*, 4 of *H. influenzae*, 2 of *E. coli*, 2 of *K. pneumoniae*, and 2 of *P. aeruginosa*.. All bacteria were isolated from patients with respiratory tract infections at the Royal Infirmary Edinburgh. The antibody levels to LPSs as determined by ELISAs were then compared between donors and the different species.
Results: The results indicated that antibody levels to LPS could be detected in all serum samples, ranging widely with the highest levels up to 5 times the median level. By regression analysis of scattergrams, comparing pairs of LPSs, antibodies to LPS from *A. baumannii* showed similar levels to *P. aeruginosa*, while those to *H. influenzae* showed antibody levels similar to those of *E. coli* and *K. pneumoniae* LPSs.
Conclusions: In the healthy population of SE Scotland it appears that healthy people are exposed to strains of *A. baumannii* at similar levels to their exposure to *P. aeruginosa*, and both species are found commonly in the environment. Similarly, exposure to *H. influenzae* was at similar levels to both *E. coli* and *K. pneumoniae* and all are common commensals. Pre-existing antibody levels specific to the LPS of these species could be directly related to susceptibility of patients to nosocomial pneumonia caused by the particular opportunist pathogen.

P1677 Extended-spectrum β-lactamase producing Enterobacteriaceae in the faecal flora of Portuguese nursing home residents

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Objectives: Our previous work in community clinical isolates alerted us for the finding of extended-spectrum β-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 20 E. β-lactamases were characterised by isoelectric focusing. 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 pI > 8 alone or in association with β-lactamases of pIs 5.4 and 7.4.
ESBL gene was successfully transferred coding a β-lactamase of pI > 8 alone or in combination with the other β-lactamases.
Conclusion: Our results showed that nursing homes are particular niches of community, acting as reservoirs of ESBL producing Enterobacteriaceae.
β-lactamase isoelectric points, alert for the hypothesis of one successful track of community dispersion of CTX-M-15, in different combination with other β-lactamases, as in the CTX-M-15 producing Portuguese ST131 *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

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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.

Virtuals and E-bugs: teaching microbiology

P1679 e-Bug: evaluation of the e-Bug educational pack in England, France and the Czech Republic

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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 sections 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 of the microbes 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.

P1680 Virtual microbiology laboratory – teaching and learning experience in the modern era

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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 lead to increased number of students from geographically diversed locations. As a result, it is increasingly difficult to organise laboratory classes with limited resources such as laboratory space and number of teachings staffs. 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, 2007/08, 2008/09; class-size ranged from 174 to 190). These web materials comprised of a series of 58 web images, 19 videos, 9 on-line 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 breath of information, it may not replace classroom or bench-side teaching where in-depth discussion are generated, hypothesizing- and theorising-skills are developed and practised.

Carbapenem resistance in Enterobacteriaceae

P1681 Emergence of multidrug-resistant *Klebsiella pneumoniae* producing KPC-type carbapenemase, Italy

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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 Klebsiellae 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.

P1682 Emergence of KPC(+) *K. pneumoniae* strains in Greece: evaluation of detection methods

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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-lactamases, 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 enterobacteriacae in routine practice. Boronic acid/imipenem disc test is safe, quick and easy to perform, in order to detect KPC(+) strains.

P1683 Rapidly rising prevalence of KPC-2-producing *Klebsiella pneumoniae* in a Greek tertiary-care hospital

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Objectives: 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, cloverleaf test and boronic acid disk tests. MICs were determined by Vitek 2 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 66.4 y, range 17–88 y) 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 hyperendemic situation.

P1684 Emergence of *Klebsiella pneumoniae* isolates possessing KPC β-lactamase in Israel, Puerto Rico, Colombia and Greece

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Background: *Klebsiella pneumoniae* carbapenemases (KPCs), are carbapenem-hydrolysing β-lactamases belonging to Bush group2f, 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 ertapenem (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.

Country	Number tested for KPC	Number positive for KPC (KPC+)	Number of sites with KPC	MIC range for KPC positive isolates (μg/mL)		
				ERT	IMI	MER
GR	11	1	1	>16	16	32
IL	14	13	4	>16	8–>32	8–>32
PR	8	8	1	0.5–>16	2–16	1–32
ZA	5	0	0	–	–	–
KR	2	0	0	–	–	–
TW	1	0	0	–	–	–
IT	1	0	0	–	–	–
AR	3	0	0	–	–	–
BR	1	0	0	–	–	–
CO	4	4	2	4–16	2–32	1–>32

P1685 Presence of the KPC carbapapenemase in *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from 6 hospitals in Colombia

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Objective: 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 were sent to the laboratory, 34 isolates of *K. pneumoniae* from 6 hospitals of different cities (Bogota: 4 hospitals, Medellin: 1 hospital, Barranquilla: 1 hospital) and 2 isolates of *P. aeruginosa* from an institution the city of Tunja. The isolates were re-identificated with MicroScan and

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 + mercaptoacetic acid (SMA) 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 imipenem and susceptibility to meropenem (1 isolate of *K. pneumoniae*), 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.

P1686 Outbreak of KPC-2-producing *Klebsiella pneumoniae* in a university hospital, Ribeirão Preto City, Brazil

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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®1 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 carabepenem 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 Tn4401, as published previously. Conjugation experiment demonstrated that blaKPC-2 gene and carabapenem-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.

P1687 Detection of KPC enzymes in *Klebsiella pneumoniae* isolates from NY/NJ sites in the TEST Program

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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 sporatic 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.

MIC (μg/mL)	ERT	MER	IMI
⩾32	na	31/31 (100)	na
⩾16	66/66 (100)	31/31 (100)	18/18 (100)
8	12/13 (92.3)	9/9 (100)	53/54 (98.1)
4	6/8 (75)	11/12 (91.6)	12/13 (92.3)

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.

P1688 Prevalence of extended-spectrum β-lactamases among Enterobacteriaceae strains from the United States (USA; 2007) and correlation with KPC carbapenemases

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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. Carbapenemase 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 those, 215 belonged to four species: *E. coli*, *K. pneumoniae* (KPN), *K. oxytoca* and *P. mirabilis*. The 72 remaining isolates were AmpC-producing

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.

Organism groups (no.)	β-lactamase types (no. of isolates)																		
	CTX-M-				SHV-										TEM-		OXA-		Other
	(15)	(14)	(2)	(3)	1	(5)	(7)	11	(12)	(27)	(30)	(31)	(40)	1	(10)	2	10	β-lactam-ases
Carbapenem-susceptible (235)	34	22	1	1	19	10	10	20	26	2	10	4	1	93	1	16	6	(PER-like)
Carbapenem non-susceptible strains (51)[a]	–	1	–	–	4	1	–	18	12	–	3	–	1	45	–	2	2	–
KRC-producing isolates (45)	–	–	–	–	4	–	–	17	11	–	2	–	1	40	–	1	1	–

1. Include 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

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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 ertapenem.

Results: Over 250 putative ESBL strains were systemically screened to evaluate prevalence of carbapenemase producing strains, but they were not detected. In contrast, a highly carbapenem resistant *Enterobacter cloacae* strain harbouring IMI-2 gene was isolated from a patient in intensive care unit. The IMI-2 positive strain was highly resistant to meropenem (MIC > 32), imipenem (MIC > 32) and ertapenem (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

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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 isoelectricfocusing (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

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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 4 mg/L, 4 mg/L, and 8 mg/L, respectively. MIC for imipenem determined by Etest for MBL detection was >32 mg/L and for imipenem plus EDTA 2 mg/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.

P1692 Emergence of pan-resistant VIM-1 producing *Klebsiella pneumoniae* in Belgian hospitals

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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-lactamase (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 >32 mg/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= 16 mg/L). Tigecycline was active against 4/5 strains (one isolate with intermediate resistance, MIC= 4 mg/L). blaVIM-1 was part of a class 1 integron that also carried aacA7, dhfr1, 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.

P1693 Systemic infection by multiresistant *Klebsiella pneumoniae*, VIM-1, TEM-1 and SHV-12 β-lactamase producer in a kidney transplant recipient patient: study of 5 isolates

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Objectives: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is an emerging nosocomial pathogen. Our aim was to evaluate the antimicrobial susceptibility profiles, the genetic relatedness and the mechanisms of β-lactam resistance among 5 clinical isolates of *K. pneumoniae* that caused infections in a renal transplanted patient throught a month.
Methods: The patient, a 74-year-old man, had a UTI after one week of a kidney transplant and levofloxacin treatment. It was changed to imipenem (Ip) after K1 isolation. K2, K3 and K4 were isolated 4 days, 1 and 3 weeks later. As K4 is resistant to Ip treatment was changed to tygecicline (Ty). One week later K5 is recovered. Susceptibility to 13 β-lactamic (BL) and to 12 non-BL agents was determined (CSLI). Extended-spectrum-BL (ESBL) and metallo-BL production were detected using synergy test. PCR of bla TEM, -SHV, -CTX, DHA-1 and-VIM was performed and VIM, TEM and SHV products sequenced. Clonal relatedness was determined by ERIC PCR and PFGE.
Results: The 5 isolates are of the same clonal group. Resistance to 10 or all of BL and to 8 of non-BL was found. Main results are shown in the table.

	KP isolates				
	K1	K2	K3	K4	K5
Origin	Urine	Urine	Urine	Urine	Blood(2)
Ip treatment*	No	Yes	Yes	Yes	No
Ty treatment	No	No	No	No	Yes
Aztreonam MIC (mg/1)	128	128	128	8	128
Ip MIC (mg/1)	0.12	2	2	64	0.12
Ty MIC (mg/l)	1	1	1	0.5	8
ESBL test (CSLI)	+	−	−	−	+
EDTA test	−	+	+	+	−
VIM-1	−	+	+	+	−
TEM-1	+	+	+	−	+
SHV-12	+	+	+	+	+

*4 or more days prior to isolation.

Conclusions: For the CRKPs (VIM-1 producers) imipenem MIC can be as low as 2 mg/l. As the presence of VIM-1 mask the presence of an ESBL when CSLI confirmatory test is done with cefotaxime or cetazidime, it should be done with aztreonam. Resistance to tygecicline is of concern. There is a relation between previous antibiotic take and resistance genes. The emergence of CRKP as a consequence of circulation of blaVIM gene must be prevented by active laboratory surveillance and carefully carbapenem use.

P1694 Detection of metallo-β-lactamases in Enterobacteriaceae clinical isolates at a university hospital

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Objectives: The aim of our study was to determine the occurrence of MBLs in isolates of the Enterobacteriaceae resistant to carbapenems that had been isolated from the University Hospital, Clinical Center of Serbia, Belgrade, during the first nine months of 2008.
Material and Methods: A total of 195 non-replicate Enterobacteriaceae isolates (47 *Proteus mirabilis* and 148 *Klebsiella pneumoniae*) were obtained from specimens of patients with nosocomial infections. MIC for imipenem and meropenem was determined by E-test (AB Biodisk,

Solna Sweden). MBL production was determined by using the imipenem-EDTA synergy test. For detection of ESBLs production, double disk synergy test was used. Multiplex PCR assay was used to detect and differentiate each of five families of MBL genes: IMP, VIM, SPM-1, GIM-1, and SIM-1.
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.

P1695 Recurrent urinary tract infections due to metallo-β-lactamase-producing *Klebsiella pneumoniae* in the community setting

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Objectives: Metallo-β-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 ipimenem-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 meropenm MICs ranged from 8 to >32 mg/l. All isolates were susceptible to aminoglycosides, aztreonam and colistin but resistant to other β-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/clavulate 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.

P1696 OXA-48 carbapenemase-producing *Klebsiella pneumoniae* in the UK

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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. β-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: Centre 1, 1 isolate from a patient transferred from Turkey; Centre 2, 5 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 ertapenem (MIC 8–>16 mg/L) and non-susceptible to imipenem (MICs 4–>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 β-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.

P1697 In vivo selection of resistance to carbapenems in SHV-5 and DHA-1 producing strains of *Klebsiella pneumoniae* in a university hospital, Plzen (Czech Republic)

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Objectives: Resistance to carbapenems in enterobacteria 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 β-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.
Methods: All non-repetitive isolates of *K. pneumoniae* resistant to carbapenems were collected during the period of January 2007 and June 2008. In these isolates, ESBL, AmpC and MBL production were preliminary identified by DDST 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 β-lactamase content, but susceptible to

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 on going.
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P1698 In vivo selection of imipenem-resistant *Klebsiella pneumoniae* producing clavulanic acid inhibited extended-spectrum β-lactamase and plasmid-encoded cephalosporinase

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Objectives: Resistance to carbapenems, while uncommon in Enterobacteriaceae, 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 β-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 β-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 blaCTXM-15 gene, plasmid-mediated AmpC β-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 β-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

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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 meropenem-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 cefotaxime and ceftazidime were considered as potential ESBL producer 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-β-lactamses 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), aztreonam (>64), cefotaxime (>256), ceftazidime (>256), cefoxitin (>64), cefpirome (>64), ertapenem (128), imipenem (4), meropenem (8), piperacillin (>64), piperacillin/tazobactam (>64), sulbactam (>2), cefotaxime/cloxacillin (>256), colistin (0.5), ciprofloxacin (>8), minocycline (32) and tigecycline (2). *K. pneumoniae* strain K1 was resistant to ertapenem (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-β-lactamases nor KPC β-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 infection 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

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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 β-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. β-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. PAβN (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.

P1701 Biological cost of resistance to carbapenems in a *Klebsiella pneumoniae* isolate

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Carbapenems (imipenem, meropenem, ertapenem) are bactericidal antibiotics that acts by inhibiting the bacterial cell wall, and which are used mainly in the treatment of severe infections. Resistance to carbapenems are exceedingly rare in Enterobacteriaceae despite its frequent use since long time ago. The objective of this study was to ascertain whether a strain of *K. pneumoniae* resistant to carbapenems have less fitness than those that are carbapenem-sensitive in competing, and would therefore tend to disappear in their competition with carbapenem-sensitive strains in the absence of antibiotics.
Methods: One carbapenem-resistant strain and six carbapenem-sensitive strains of *K. pneumoniae*.
Thirty competition experiments by pairs of strains were conducted, 5 by each sensitive strain (carbapenem-R versus carbapenem-S). The experiments were performed in nutrient broth (NB). Equal amounts of the strains were challenged (approx. 50% and approx. 50%) for 5 days, with a daily change to a new medium. Six differential counts were performed (days 0, 1, 2, 3, 4 and 5). For the differential counts on the fifth day, MacConkey agar with and without ertapenem (2 mg/L) and a replica plater machine were used.
The mean of results for each pair of strains and the range, in parenthesis, are presented below.
Results: In all the pair experiments there were a relative increase in the carbapenem-sensitive strain that translated into a count of 76% (63–90%), 94% (90–98%), 80% (71–95%), 93% (78–99%), 83% (78–89%), and 95% (89–98%) on the fifth day.
Conclusions:
- Resistance to carbapenem entails a biological cost (less fitness) in all the experiments by pairs for the *K. pneumoniae* strain assayed.
- This biological cost hinders their competition with carbapenem-sensitive strains in the normal intestinal flora, which would make them less likely to cause infections.

Acinetobacter baumannii: resistance

P1702 Proteomic analysis of the secretome of *Acinetobacter baumannii*

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Objectives: To identify extracellular proteins secreted by different strains of *Acinetobacter baumannii* which could be potentially responsible for the disease, using a proteomic approach.
Methods: The proteomic study was performed in nine *A. baumannii* clinical isolates previously identified by ARDRA and classified into different clones by PFGE. The isolates caused a nosocomial outbreak (3 strains), sporadic infectious diseases (4 strains) and community-acquired diseases (2 strains). Antimicrobial susceptibility tests were performed by E-test. To obtain the proteins, the isolates were grown in minimal medium plus casaminoacids to an OD600 = 0.6. The cells were harvested by centrifugation; the supernatant was filtered with 0.22 μm filters and concentrated with Amicon Tubes (50 ml). Protein analysis was performed by 12 to 20% polyacrylamide gradient gel electrophoresis with SDS. Characterisation of the proteins was done by MALDI-TOF/TOF mass spectrometry analysis.
Results: Nine isolates of *A. baumannii* were studied. Seven were resistant to all antimicrobial agents but colistin and the other two strains were susceptible to meropenem, imipenem, ciprofloxacin, piperacillin and aztreonam. Among the proteins characterised the following were found: 1.The presence of a 45Kda protein OmpA, a 25Kda alkali-inducible disulfide interchange protein, the putative toluene 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 carbapenems, 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.
Conclusion: The identification of secreted proteins provides a target for an anti-*Acinetobacter* vaccine and drug development as well as for further investigation on the pathogenicity of the infections by *A. baumannii*.

P1703 Multi-centre study of dissemination and diversity of carbapenem-resistant *Acinetobacter baumannii* Spanish clinical isolates

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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 period (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 (tigecylcine 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 are 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 isolates (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* multirresistant 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

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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 API32GN 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 revelead 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

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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: Thirteen OXA-40-Ab-producers were selected from a collection obtained during 2001–2007 in 2 Portuguese hospitals. An OXA-40-producig Ah and one imipenem-susceptible Ab (ISAb) isolate that did not present any acquired oxacillinase, were also included.
Chromossomal and/or plasmid location of blaOXA-40 was determined by I-CeuI 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-CeuI 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 Ah 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, pACICU1) in the regions following the 3′ 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 Ah 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

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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. ISAba1 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. ISAba1 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

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Objectives: *Acinetobacter baumannii* is an important nosocomial pathogen which is often associated with multiple drug resistance. First imepenem resistant *Acinetobacter baumannii* isolates in Latvian hospitals were detected in fall of 2007 and afterwards rapidly spread

throughout the country. The aim of this study was to characterise isolates both by their phenotype and genotype.
Methods: 109 *Acinetobacter baumannii* isolates were collected from three biggest Latvian hospitals in Riga. All isolates were identified by BD Crystal E/EN. Antibiotic resistance was determined by disc susceptibility tests according to CLSA standards. Presence of the OXA-51 like and OXA-23 like elements was established by PCR (Turton et al., 2006). All strains were typed by ERIC-PCR and using DiversiLab (Biomerieux). MLST was done as described by Bartual et al., 2005.
Results: All *Acinetobacter baumannii* isolates showed resistance to imepenem and with some minor differences were also resistant to other antibiotics (amikacin, ampicillin-sulbactam, cefepime, ceftazidine, gentamicin, piperacillin-tazobactam and trimethoprim-sulfamethoxazole). All isolates were carrying OXA-51 like element and 103 isolates also possessed OXA-23 like element. Both typing methods – ERIC-PCR and DiversiLab did not reveal any differences in the chromosomal background of the tested strains indicating that they belong to the same strain. MLST of several arbitrary chosen isolates resulted in identical sequences and revealed a new sequence type (compared to ones in mlst.zoo.ox.ac.uk) as on December 2008.
Conclusion: A new sequence type imepenem resistant *Acinetobacter baumannii* strain has become endemic in three hospitals in Riga, Latvia.

P1708 Detection of the OXA-58 carbapenemase in clinical isolates of *Acinetobacter baumannii* from Cochabamba, Bolivia

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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 difussion method following the CLSI recommendations. Antibiotics tested were amikacin, cotrimoxazole, cloranphenicol, amoxicillin/clavulanic acid, piperacilline, imipenem, meropenem, ceftazidime, cefepime, cefotaxime, cefexime, ciprofloxacine, and levofloxacine. Clonal relatedness was performed by PCR-fingertyping, plasmids were determined by a commercial kit, and class 1 integrons and insertion sequences (ISAba 1, ISAba 2 and ISAba 3) by PCR experiments with the corresponding primers, (integrons were also digested with HaeIII and EcoRI). OXA-type carbapenemases (-23, -40, -51 and -58) were detected by Multiplex PCR. Sequencing experiments were done with OXA-58 carbapenemase positive isolates.
Results: The resistance results showed that 7 isolates were resistant to most of the antibiotics tested, mainly to imipenem and meropenem. Typing experiments showed 5 different genotypes, one of them (named Bol1) including 5 isolates. Class 1 integrons were detected in all isolates belonging to clone Bol1, ranging in size between 540 to 780 bp. Multiplex-PCR and sequencing analysis showed the presence of the OXA-51 and OXA-58 carbapenemases in all isolates from clone Bol1. All of them also contained IS Aba 3 and two were positive for ISAba 1. ISAba2 was present in an isolate from other clone. We found clear relationship between OXA-58 gene and ISAba 3. Plasmid analysis and hybridisation with an OXA-58 probe detected the relation of the carbapenemase gene with different structures.
Conclusions: This is the first description of the OXA-58 carbapenemase in isolates of *A. baumannii* from Bolivia. The detection of the enzyme in a multiresistant clone, named Bol1, and its relationship with plasmids is of great concern as it means the possibility of spreading carbapenem resistance amongst the hospitals of the country.

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

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Objectives: To investigate the mechanism of stepwise acquired multi-drug 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, fluoroquinolones, piperacillin and piperacillin-tazobactam and sensitive to colistin. 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).

Strain	MIC (μg/ml)					
	TGC	IPM	MPM	SXT	TOB	LEV
A009703	4	32	32	40	⩽1	8
A009747	4	32	32	40	⩽1	8
A009808	4	32	8	40	⩽1	8
A009974	4	32	32	40	⩽1	8
A010018	4	32	32	40	⩽1	8
A010068	4	32	16	40	⩽1	8
Antimicrobial therapy of amikacin, ciprofloxacin and co-trimoxazole						
A010324	16	>32	>32	⩾320	⩽1	>8
A010416	16	>32	>32	⩾320	⩽1	>8
A010482	16	>32	>32	⩾320	⩽1	>8
A011650	16	>32	>32	⩾320	8	>8
A012428	16	>32	>32	⩾320	⩽1	>8
F015146	16	>32	32	⩾320	8	>8

Two later isolates also showed resistance to tobramycin. Tigecycline-resistant isolates showed a >100-fold increase in adeB mRNA transcripts compared to tigecycline-sensitive isolates. There was no increase in adeJ transcripts.
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

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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 glycylcycline antibiotic with broad-spetrum activity that appears to be the only new

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), ceftazidime (CAZ), imipenem (IMP), aztreonam (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* bawmannii. 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, bacteraemic patients. Colistin and tigecycline remain the only active agents towards such strains, according to our results.

P1711 Genetic basis of resistance to aminoglycosides in *Acinetobacter* species and spread of armA in *Acinetobacter baumannii* sequence group 1 in Korean hospitals

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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* genomic species 13TU isolates (n = 14). Genes encoding aminoglycoside-modifying enzymes, ant(3″)-Ia, aac(6′)-Ib, aph(3′)-1a, 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* genomic species 13TU, whereas armA is responsible for the high-level resistance to aminoglycosides in *A. baumannii* sequence group 1.

P1712 Antibiotic resistance pattern of *Acinetobacter* species in Kuwait hospitals

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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.
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, cefotaxime, ceftazidime, cefepime and piperacillin-tazobactam were each 256 ug/ml, while MIC50 and MIC90 of imipenem, meropenem, tigecycline and colistin were 1.5 and 32, 3 and 32, 0.38 and 3, 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

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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(ant2), aph3–3, aph3–6, ant4–1, ant4–2, 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-aminoglycosie 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

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Objectives: Nosocomial infections caused by multidrug-resistant *Acinetobacter baumannii* (MDRAB) have been increasing in recent years, posing serious threat to public health worldwide.

Methods: The susceptibility to 9 antimicrobial agents of 35 clinical *A. 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 *A. baumannii* isolates. Furthermore, mutation analyses of gyrA or 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 *A. 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'CS-blaIMP-1-aadA4–3'CS, 5'CS-aacA4-aadA1–3'CS, and 5'CS-aacC1-aadA1–3'CS.
Conclusion: The expression of adeB gene was associated with resistance to ciprofloxacin and ampicillin/sulbactam in *A. baumannii*. Multiple mutations in gryA and parC also played a role in ciprofloxacin resistance. The major metallo-β-lactamase contributing to imipenem resistance in *A. baumannii* in Taiwan was blaIMP-1, which was carried by class 1 integron. Class 1 integron was associated with MDR phenotype to *A. baumannii*.

P1715 Multiplex PCR for rapid detection of genes encoding Oxa and metallo-β-lactamases in *Acinetobacter* spp.

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Acinetobacter spp, resistance to carbapenems, have become common in hospitals worldwide. Carbapenem resistance mechanisms described in *A. baumannii* include hydrolysis by β-lactamases, alterations in outer membrane proteins and penicillin-binding proteins, and increased activity of efflux pumps. However, carbapenemases, such as metallo-b-lactamase (MBL) or oxacillinases, are the most concerning.
Objective: The present study aimed to develop a multiplex PCR assay to detect and differentiate alleles coding oxacillinase and MBL in nosocomial carbapenem resistant *Acinetobacter* spp.
Material: The study included a total of sixty-eight isolates of *A. baumannii*, sixty four carbapenem-resistant and four carbapenem-susceptible strains. These isolates were obtained from 4 different hospitals of the state of São Paulo, Brazil. The following reference strains were used in this study: *P. aeruginosa* producing IMP-1, VIM-1, SIM-1 enzymes and *A. baumannii* producing Oxa-23 and Oxa-25 enzymes as positive controls and *A. baumannii* ATCC 19606 was used as a negative control. The isolates were identified by API (NE) (Biomerieux-France). Minimum inhibitory concentrations (MICs) of carbapenem were determined by broth microdilution and interpreted using Clinical Laboratory Standards Institute (CLSI) breakpoints. MBL ETEST was performed following the manufacturer's recommendations (AB Biodisk (North America Inc., N.J.) to confirm the MBL expression phenotypically in these strains.
Amplification by polymerase chain reaction with oligonucleotide primers specific for ISAba1 region was performed as described elsewhere
Results: Among, 64 carbapenem-resistant *A. baumannii* strains isolated from 4 Brazilian hospitals, blaoxa-23-like was present in 22 and IMP-1 only in 4 isolates. ISAba1 was identified in all carbapenem resistant strains except one. The imipenem MIC among blaoxa-23-like positive strains ranged from 16 to 128 ug/ml and was lower compared with IMP-1 positive strains (MIC > 128 ug/ml). Phenotypically expression of metallo-β-lactamase was confirmed by MBL-ETEST in all 4 IMP-1 positive strains.
Conclusion: The multiplex PCR results detected and distinguished alleles coding oxacillinase and MBL carbapenemases in carbapenem resistant *A. baumannii* and was consistent with the previous single PCR assays and could be an useful tool to understanding the dissemination of resistance of this microorganism in the hospital setting.

P1716 Multidrug-resistant *Acinetobacter baumannii*: clinical impact at a university-affiliated hospital

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Objectives: To evaluate impact of multidrug resistant *A. baumannii* (MRAB) on clinical outcome of patients admitted at a university affiliated hospital.
Patients and Methods: Observational and prospective study of a cohort of non-paediatric patients with MRAB isolates. Data collection from clinical records was done according to a standard protocol. We analysed epidemiological, clinical, microbiological and laboratory data from January 2007 through June 2008. Patients with MRAB were identified by review of results of microbiology cultures from the hospital microbiology laboratory. *A. baumannii* isolation, identification and sensitivity test (VITEK-2; Biomerieux, France) were performed by standard criteria. Mortality was assessed till 30 days. Colonisation was defined as positive culture but no clinical symptoms or signs of infection.
Results: 101 patients with 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 underline conditions were high blood presure (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% piperacilin-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% aminoglycoside, colistin and tigecycline susceptible but ampicillin-sulbactam resistant and 20% only colistin and tigecycline 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 treatmets and dicreased associated mortality and morbility.

P1717 Ten-year resistance trends of nosocomial *Acinetobacter* spp. in Russia

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Objectives: To assess the trends in antimicrobial resistance of nosocomial *Acinetobacter* spp. in Russia over the period of 1997–2007.
Materials and Methods: A total of 997 consecutive non-duplicate nosocomial isolates of *Acinetobacter* spp. were collected as part of the national surveillance studies in Russia during the following periods: 1997–1999 (n = 203), 2002–2004 (n = 464), and 2006–2007 (n = 330). The susceptibilities of isolates to various antimicrobial agents were determined by agar dilution method and interpreted according to EUCAST clinical breakpoints.

Results: The antimicrobial susceptibility of *Acinetobacter* isolates is summarised in the Table.

Antibiotic	MIC, μg/ml		% of isolates		
Years	50%	90%	S	I	R
Imipenem					
1997–1999	0.5	1	97.0	0.5	2.5
2002–2004	1	2	93.9	3.9	2.2
2006–2007	1	2	95.5	2.4	2.1
Amikacin					
1997–1999	2	16	88.6	2.0	9.4
2002–2004	128	256	33.6	1.3	65.1
2006–2007	256	⩾512	20.6	0.3	79.1
Gentamicin					
1997–1999	32	⩾256	31.0	–	69.0
2002–2004	128	⩾256	11.2	–	88.8
2006–2007	128	⩾256	15.8	–	84.2
Netilmicin					
1997–1999	ND	ND	ND	ND	ND
2002–2004	ND	ND	ND	ND	ND
2006–2007	4	16	78.2	13.9	7.9
Ciprofloxacin					
1997–1999	1	32	69.0	–	31.0
2002–2004	64	⩾128	26.3	–	73.7
2006–2007	⩾128	⩾128	8.5	–	91.5
Colistin					
1997–1999	ND	ND	ND	ND	ND
2002–2004	ND	ND	ND	ND	ND
2006–2007	0.5	1	100.0	–	0.0

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.

P1718 *Acinetobacter baumannii* 2002–2007: mathematical model insights on imipenem non-susceptibility and multi-class resistance

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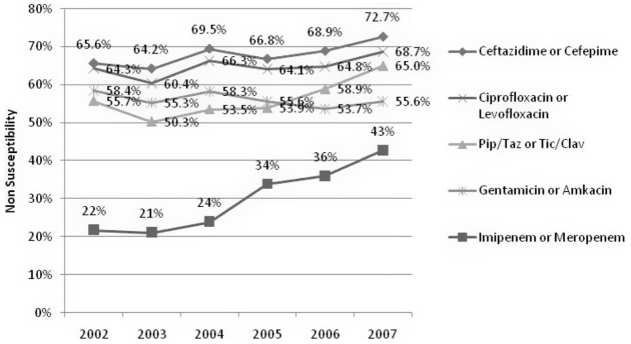
Objectives: To use a hospital based mathematical model of *A. baumannii* to provide insights for the statistical analysis of a surveillance study.

Methods: A compartmental mathematical model at the hospital level was created to investigate the changes over time of *A. baumannii* imipenem and multi-class resistance. The model was calibrated using 48,551 US isolates from the TSN Network® surveillance database (Eurofins Medinet) for the period 2002–2007. A multivariate nonlinear model was fitted to the data using the insights from the mathematical model. Covariates available for analysis were time, age, sex, state, location (Ward or ICU) and source (blood, sputum, etc). Antimicrobial classes included were carbapenems, cephalosporins, aminoglycosides, fluoroquinolones and β-lactamase inhibitors. Multi-class or multiple resistance was defined as non susceptibility to three or more classes.

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

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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 Cefoperazone-sulbactum 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

P1720 Prevalence and antimicrobial susceptibility patterns of methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecium* causing bloodstream infections in European hospitals (2005–2008)

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Objective: To evaluate the prevalence and antimicrobial susceptibility (S) of MRSA and vancomycin-resistant *E. faecium* (VREFM) in European hospitals. These organisms are usually multidrug-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 (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 coagulase-negative staphylococci were oxacillin-R.

Organism (no. tested)	MIC_{90} (mg/L) / % susceptible				
	Daptomycin	Vancomycin	Teicoplanin	Q/D[a]	Linezolid
MRSA (1,086)	0.5/100.0	1/100.0	⩽2/100.0	1/99.8	2/99.9
VREFM (134)	2/99.3	>16/0.0	>16/41.8	>2/73.1	2/98.5

[a] Q/D = quinupristin/dalfopristin.

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 episodes 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

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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

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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 eryrthromycin, 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

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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* pharmaco- and population-dynamic approaches in a community of individuals stratified according to their colonisation and β-lactam-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 the population, different patterns of use could lead to markedly different resistance distribution and prevalence.
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

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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* bacteraemia 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 bacteraemia and meningitis. Of 51 patients with bacteraemia, the most common diagnosis was pneumonia (74.6%), followed by primary bacteraemia (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* (PSSP), 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 PSSP and PRSP. In contrast to the observation in patients with bacteraemia, cefotaxime-susceptible, -intermediately resistant, and -resistant *S. pneumoniae* were noted in 72%, 14%, and 14%, respectively. All isolates from patients with bacteraemia 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

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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.

	2002	2004	2006/06	2006/59	2008/06
Penicillin	55.56	56.45	37.25	57.58	51.56
Amoxicillin	22.22	53.23	29.41	57.58	53.13
Cefotaxime		50.00	25.49	48.48	40.63
Tetracycline	29.63	30.65	27.45	42.42	42.19
Chloramphenicol	11.11	12.90	5.88	24.24	50.00
Erythromycin	66.67	66.13	50.98	63.64	73.44
Clindamycin	22.22	27.42	21.57	33.33	18.75
Spiramycin		54.84	33.33	48.48	54.69
Lincomycin	22.22	17.74	11.76	24.24	42.19
Telithromycin		12.90	1.96	0.00	34.38
Ciprofloxacin	18.52	56.45	66.67	51.52	100.00
Levofloxacin	3.70	6.56	9.80	12.12	38.10
Moxifloxacin		8.20	7.84	12.12	49.21

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 Sp resistance to 42%. Erythromycin resistance in the SP isolates (45% in AM, 68% in N) is in 2006 also similar to the resistance rate in VGS isolates (51% in AM, 64% in N) and resistance increased in 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.

P1726 Diverse populations of fluoroquinolone nonsusceptible group A streptococci recovered from colonisation and infections in Portugal (1999–2006)

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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 HinfI and LweI. 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 ST382/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 (ST382) 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).

P1727 Trends in invasive group A streptococci in adult patients in Barcelona

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Objective: The aim of this study was to analyze the invasive disease due to *Streptococcus pyogenes* (GAS) in adults patients during a ten-year period (1998–2007) in Barcelona.
Methods: All invasive GAS episodes detected in our hospital were collected. Antibiotic susceptibility was studied by microdilution. Molecular typing was performed by PFGE (SmaI) and emm-typing and selected strains were studied by MLST.
Results: A total of 127 episodes of invasive GAS disease were observed during the study period. Of them, 76 occurred in men (60%). The medium age was 57 (SD19.7, range 18–98). The overall incidence of invasive GAS disease was 2.2 episodes per 100,000 inhabitants. Among people older than 65 the invasive GAS disease (6.4/100,000) was higher than those observed among adults aged 18–64 years (1.4/100,000, $p < 0.001$). The sources of invasive GAS were blood 110 (87%), joint fluid 9 (7%), pleural fluid 7 (6%), and ascitic fluid 1 (0.8%). Most of invasive diseases were related to soft-tissue infections. The antibiotic resistance rates were as follows: erythromycin 20.5%, clindamycin 12.6%, tetracycline 17% and ciprofloxacin 2%. All strains were susceptible to penicillin. Ninety-five strains were available for molecular typing. The six most frequent emm types and their related sequence-types (STs) were emm1-ST28 (31%), emm59-ST172 (10%), emm28-ST52 (6%), emm4-ST39 (5%) emm12-ST36 (5%) and emm25-ST350 (5%).
Conclusion: The majority of invasive infections caused by *Streptococcus pyogenes* in adult patients in our area were related to soft-tissue infections and were associated with emm1-ST28 type, whereas the emm59-ST172 and emm25-ST350 types were mainly associated with injection drug users.

P1728 Phenotypes and genotypes of erythromycin-resistant group A streptococci in Barcelona

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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 (SmaI) 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 (SD19, 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%8) were related to Tn916-family of transposons (Tn6002 and Tn1545). Three GAS with ermTR gene were resistant to tetracycline related to tetM gene probably harboured by 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.

P1729 Oropharyngeal colonisation by group A streptococci in Portugal: an eight-year surveillance study (2000–2007)

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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 (0–6 yrs) in day-care centres (DCC), 2337 from school-aged children (7–16 yrs)

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 OC rates (>10%) were usually detected during winter periods (17% in 2001, 13% in 2002/03, 12% in 2007), and occasionally, during autumn of 2003 (15%) and spring of 2001 and 2004 (15% and 18%, respectively). E resistance was 10% in 2000–02, 28% in 2003, 20% in 2004, 3% in 2005, 14% in 2006 and 10% in 2007. Da resistance was lower than 10%, except in 2006 (14%). Six out of 98 PFGE types accounted for 49% of isolates studied (n = 380/775) which were included in five ST or lineages: ST36 (T12/emm12/PFGE.AB;PFGE.AP) (n = 172, mainly E susceptible); ST28 (T1/emm1/PFGE.X) (n = 87, all E susceptible); ST406 (T3.13.B3264/emm3/PFGE.BG) (n = 48, all E susceptible); ST382 (T6/emm6/PFGE.AD) (n = 38, all E susceptible); and ST39 (T4/emm4/PFGE.CZ) (n = 35, all E resistant). Three lineages and four PFGE types prevailed during peaks of OC: ST406 (PFGE.BG) in 2001 (winter and spring); ST36 (PFGE.AB) in 2002 (winter) and 2003 (autumn); ST36 (PFGE.AP) in 2003 (winter); and ST28 (PFGE.X) in 2004 (spring).

Conclusions: The GAS carrier state was seasonal and variable with age and DCC attendance. A very heterogeneous population of GAS colonised healthy carriers during 2000–07. However, some lineages/clones either resistant or susceptible to macrolides were identified as putative poor colonisers and others as widely disseminated and persistent over time.

P1730 Nocardiosis over 20 years in a tertiary care hospital

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Objectives: The aim of the study was to review and characterise the cases of nocardiosis identified in our institution between January 1989 and December 2008.

Methods: Retrospective analysis of all cases with emphasis on clinical presentation, diagnosis, treatment and outcome. Analysis of the antimicrobial susceptibility patterns of all the identified strains.

Results: During the study period 28 cases were identified; 25 were considered infections and included for analysis and 3 cases were not included because they were considered as colonisation 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%; 32% received steroid therapy within one month before the diagnosis of *Nocardia* infection. Clinical presentations were pulmonary in 72% followed by cutaneous 12%, cerebral 8%, disseminated 4% and articular 4%. The species isolated were *N. asteroides* in 52%, *N. asteroides* complex in 16%, *N. farcinica* 8%, *N. nova* and *N. brasiliensis* 4% in each and *Nocardia* spp. in 16% of cases. Clinical outcome was cure or improvement in 80%, treatment failures in 4%, relapses in 4% and death in 12%.

Sensitivity tests showed that amikacin was active in 20/20 of tested strains, imipenem in 20/22, trimethoprim-sulphamethoxazole (TMS) in 19/22, minocycline in 7/7, ciprofloxacine in 8/13 and ceftriaxone in 6/9. Two tested strains were sensitive to linezolide; one strain was sensitive to imipenem but resistant to ertapenem. TMS was the initial antibiotic treatment in 15 patients. In 6 cases (40%) it was switched to another treatment due to intolerance or lack of efficacy.

Conclusions: *Nocardia* infection is increasing in our centre. It must be considered an opportunistic infection in our centre since most of the 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

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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 UltraCleanTM Microbial DNA Isolation Kit. For rep-PCR, DNA was amplified using the *Clostridium* Kit for DNA Fingerprinting. 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.

P1732 A 20-month survey of molecular characterisation and antimicrobial susceptibilities of *Clostridium difficile* isolates in a Chinese university hospital

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Objectives: *Clostridium difficile* infection (CDI) is the leading cause of nosocomial diarrhoea and the number of outbreaks has risen markedly since 2003. However, there is still a paucity of data on CDI in China. A 20-month study of CDI was conducted at the University Hospital Huashan in order to characterise the clinical isolates of *C. difficile*.

Methods: 136 *C. difficile* isolates recovered between 2007 and 2008 were analyzed. The presence of tcdA, tcdB, binary toxin genes and tcdC deletion nt 117 was detected by polymerase chain reaction (PCR), toxin B was reconfirmed by the cytotoxicity neutralisation assay. The minimum inhibitory concentrations (MICs) of 12 antimicrobial agents were determined using the agar dilution method according to Clinical Laboratory Standard Institute (CLSI).

Results: Of 136 isolates, 104 were from the first episode of CDI, 11 were from relapses, 3 were from reinfections, and 18 were toxin-negative. Of the 107 nonrepeated and toxigenic isolates, 76 were A

(+)B(+), 29 were A(−)B(+), and 2 were A (+)B(+)Binary toxin(+). No TcdC nt 117 deletion was detected. Twenty-three different PCR ribotypes were identified with a specific clone (SH II) accounting for 18.5% of the isolates. None of them belonged to ribotype 027 or 078. All strains were susceptible to metronidazole, vancomycin and piperacillin/tazobactam. 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/clindamycin, 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.

Conclusion: 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.

P1733 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

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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.

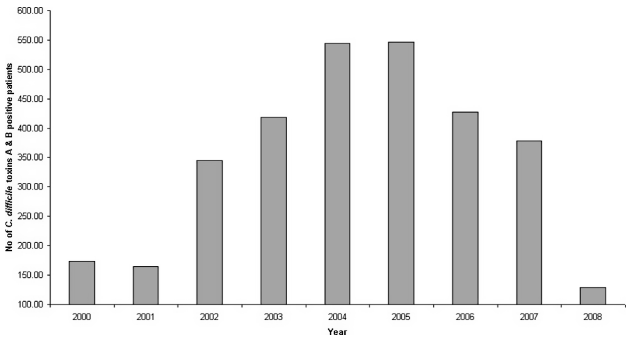

Figure 1. No. of *C. difficile* toxins A and B positive patients from 2000 to 2008.

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.

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Antifungal therapy

P1734 European Surveillance of Antimicrobial Consumption (ESAC): outpatient antimycotic use in Europe

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Objectives: To assess the total outpatient systemic antimycotic use in Europe and to identify the antimycotic 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 antimicrobial 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 antimycotics 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 antimycotic 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 DID in Belgium) and the country with the lowest (0.26 DID in Slovakia) use. Terbinafine, itraconazole, and fluconazole were the most prescribed substances, and represented more than 96% of the total outpatient antimycotic 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 antimycotic use in 8 out of 11 countries (not in Latvia, Hungary and Italy).

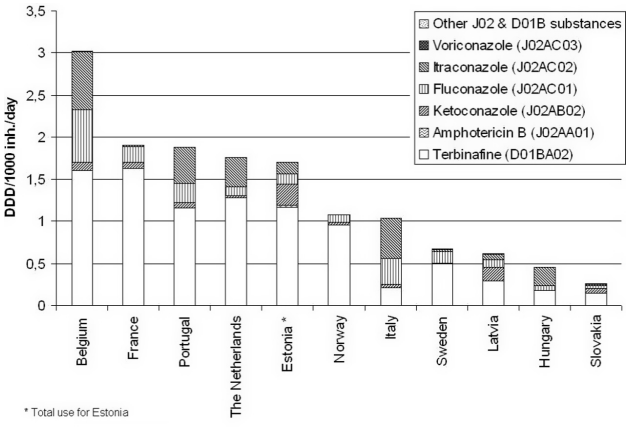

Figure: Total outpatient systemic antimycotic use in 11 European countries in 2007.

Conclusion: Our study demonstrates a variation of outpatient systemic antimycotic use in Europe as striking as that of outpatient systemic antibiotic use. The ESAC data facilitate auditing of antimycotic prescribing and evaluation of the implementation of guidelines and public health policies to promote its judicious use.

P1735 Management of candiduria: an interview schedule

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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 (199 internists, 79 surgeons, 27 intensivists) specialists. Of the participants, 215 (54.7%) were male, 178 (45.3%) were female 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: The approach of Infectious Diseases (ID) and other specialists to candiduria

	ID specialists n = 88 (%)	Other specialists n = 305 (%)	Statistic p
Mean age (year)	38.28±8.4 (24–60)	33.12±8.1 (24–63)	
Mean time after specialisation training	7.72±7.85 (min 0 – max 30)	3.58±6.37 (min 0 – max 28)	
Following related guidelines	65 (73.9)	59 (19.3)	
Second urinary culture	**74 (84)**	**130 (42.6)**	**0.0000***
Starting of therapy without second culture result	25 (33.7)	24 (18.4)	**0.013***
Starting of therapy according to second culture result	49 (66.3)	106 (81.5)	
Starting of therapy in high risk patient when second urine culture is positive	66 (89.1)	87 (66.9)	**0.0004***
Changing of the urinary catheter	55 (74.3)	95 (72.5)	0.849
Searching for another fungal infection focus	66 (89.1)	105 (80.7)	0.116
Choice of therapy for patients with neutropenia			
Fluconazole	29 (39.2)	39 (29.7)	
Other antifungal agent	45 (60.8)	91 (70.3)	
Choice of therapy for patients without neutropenia			
Fluconazole	72 (97.3)	116 (89.2)	
Other antifungal agent	2 (2.7)	14 (10.8)	

P1736 Empirical antifungal therapy in selected patients with persistent febrile neutropenia

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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 in clinical criteria and 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%). EAT was indicated in thirty two episodes (60.4%) with a median of duration of 10 days (range: 2–164); in the rest of episodes (n = 21) EAT was no indicated. The overall IFI incidence was 17% (n = 9). In the group that received EAT, nine patients developed IFI (28.1%), in comparison with no-one patient in the group that did not receive EAT. The 30 days-global mortality was 18.9%, 28.1% in the group that received EAT and 4.8% in the group that did not received it. The IFI-related mortality in the group that received EAT was 6.2% and 0% in the group that did not received EAT.
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 over-treatment.

P1737 Outcomes associated with antifungal treatment and fluconazole treatment failure for candidaemia or invasive candidiasis in patients admitted into intensive care units

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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 were selected from January07 to April08 on the following criteria: primary or secondary diagnosis using ICD-9 codes for candidaemia or IC receiving any IV antifungal drug (AF), age $\geqslant$18, and admitted in ICU. Patients were classified in two groups: those who started with fluconazole (FLC) and those who started with any other licensed AF therapy (OLAT). FLC success was considered when no second-line AF was needed. Final clinical evolution was registered. Hospital length of stay-LOS (pre-ICU, ICU, Post-ICU) and resource use (ventilation assistance, central catheter, second-line AF) was calculated from the start of AF until discharge from ICU or death.
Results: A total of 43-patients met inclusion criteria, 36 of whom started on IV FLC, and 7 on OLAT (4 caspofungin, 1 AmB-L, 1 AmB-LC, 1 voriconazole). Mean age (SD) was 61.8 (14.7) years in the FLC arm vs. 46.9 (16.5) in the OLAT ($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.

57.1% ($p < 0.05$), respectively. Apache score (SD) was superior in the OLAT, 16 (10) vs. 13.7 (7.1) in FLC, $p < 0.01$. IC was mostly diagnosed (66.7%) in FLC arm while candidaemia were diagnosed (57.1%) in OLAT arm. *C. albicans* was isolated in the 52.8% of FLC arm, while *C. parapsilosis* was isolated in 42.9% of OLAT. Mean (SD) first-line AF treatment duration was 17.3 (23.6) days in the FLC arm vs. 9.4 (11.6) days in OLAT arm ($p < 0.01$). Mean LOS and resources use was superior in the FLC arm ($p < 0.01$). When considering FLC arm alone, it was considered success in 58.3% of patients, while failed in 41.7%. Final clinical evolution were failure (36.1%) and unclassified (36.1%) in FLC vs. failure (57.1%) in OLAT ($p < 0.01$). Mortality rates were 52.8% in FLC arm vs. 85.7% in OLAT arm ($p < 0.01$), mostly due to invasive fungal infections+other reason.

Conclusions: Over all 37% of patients required second-line AF therapy. Mortality rates due to the fungal infection were higher in the OLAT arm thereby resource use was slightly lower versus the FLC arm. No adjustment was made due to being an interim analysis but further analysis will be required.

P1738 The effect of an antimycotic restriction programme on mortality rate and incidence of candidaemia at a cardiothoracic intensive care unit

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Objective: In recent years echinocandines are likely to replace fluconazole as empiric antifungal therapy among intensive care patients. However to reduce costs, an antimycotic restriction program was initiated at our institution. To compare the effect of antifungal restriction on the mortality rate and incidence of candidaemia, a prospective study was performed at the cardiothoracic intensive care unit (ICU).

Methods: All patients admitted at the cardiothoracic ICU between December 2006 and 2008 for at least 4 days were enrolled. Before the 25th of October 2007 the choice of any antifungal treatment was left to the discretion of the treating physicians at the cardiothoracic ICU. Thereafter the use of systemic antifungal treatment except for fluconazole was restricted to the guidance by a specialist for infectious diseases. The outcome of the ICU patients and the incidence of candidaemia were analyzed.

Results: In the first study period 23 (27.7%) and 4 (4.8%) out of 83 patients died or developed candidaemia caused by *Candida albicans* in 2 patients and *Candida parapsilosis* in 3 patients during admittance at the ICU. Caspofungin was administered as predominant antimycotic treatment in 42 patients for a total time of 642 days, whereas fluconazole was only given in 5 patients for a total time of 83 days. After implementation of the antifungal restriction policy, the overall use of empiric antimycotic treatment was significantly reduced in the ICU population ($p = 0.004$), and fluconazole was administered as initial antifungal treatment in the majority of patients (29 out of 107 patients for a total time of 424 days), followed by caspofungin in 8 patients for a total time of 130 days. In the second study period candidaemia due to *Candida albicans* in 4 patients, *Candida lusitaniae* and *Candida parapsilosis* in 1 patient was detected in 4.6% of the ICU patients and the overall mortality rate was 20.4%. Antimycotic costs could be reduced from 28.9 Euros per patient and day of ICU stay to 14.9 Euros per patient and day after implementation of the antimycotic restriction program.

Conclusion: Similar mortality rates were found in the cardiothoracic ICU population before and after restriction of systemic antifungal treatment. Fluconazole was still effective as early antifungal empiric and preemptive treatment in the ICU population because *Candida albicans* was the predominant candidaemia species.

P1739 Decreasing candidaemia rate in abdominal surgery patients after introduction of prophylactic and early presumptive therapy with fluconazole

B.J. Holzknecht, J.D. Knudsen (Hvidovre, DK)*

Objectives: Candidaemia in intensive abdominal surgery patients has been of increasing interest recently and antimycotic prophylaxis is now widely recommended for these patients. In 2007 fluconazole was added 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 candidaemias and bacteraemias 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.

P1740 Liposomal amphotericin B in intensive care unit patients previously treated with fluconazole: a retrospective, efficacy and safety multi-centre study

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Objective: Efficacy and safety of Liposomal amphotericin B (L-AmB) in intensive care unit (ICU) 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 (49%) 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%)

C. albicans (54%) *C. parapsilosis* (15%) and *Aspergillus* spp (12%). Most common reasons for choosing L-AmB were: severe sepsis or septic shock (61%) L-AmB spectrum including suspicion of filamentous fungi (56%) guidelines application (42%) and infection localisation (24%). Median *Candida* score was 4.0 and median Sevilla score was 11.0. Mean duration of L-AmB treatment was 15.2 days and mean dose was 4.2 mg/kg/day. Satisfactory clinical response (complete and partial response) was achieved in 49% (95% CI:34, 64) of patients and microbiological response (negative culture) in 44% (95% CI:29, 59) of patients in the intention to treat analysis. Within evaluable patients, satisfactory clinical and microbiological responses were: 61% (95% CI:44, 77) and 82% (95% CI:66, 98), respectively. Eight treatment-related AEs were reported, but only 1 was serious: a case of renal failure requiring a change in antifungal treatment. There was no change in the overall mean creatinine value at the end of treatment in the patients treated with L-AMB, despite the fact that 51% of the patients were receiving nephrotoxic drugs concomitantly.

Conclusion: L-AmB as second line treatment after fluconazole showed high clinical and microbiological response in evaluable patients. L-AmB was well tolerated even in patients with concomitant nephrotoxic drugs. L-AmB was mainly selected for patients with severe sepsis or septic shock and also due to its broad spectrum activity. L-AmB can be considered an effective and safe option both in empiric and confirmed IFIs in critically ill patients after fluconazole treatment.

P1741 Continuous infusion of amphotericin B deoxycholate in the treatment of cryptococcal meningoencephalitis: analysis of safety and fungicidal activity

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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 cerebrospinal fluid (CSF) obtained from lumbar punctures in days 0, 3, 7 and 14 of treatment. We monitored patients in hospital for 2 weeks. At this time of study, 6 patients have been included.

Results: Analysis of data on 6 patients has shown that all patients presented a progressive reduction in CSF 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.

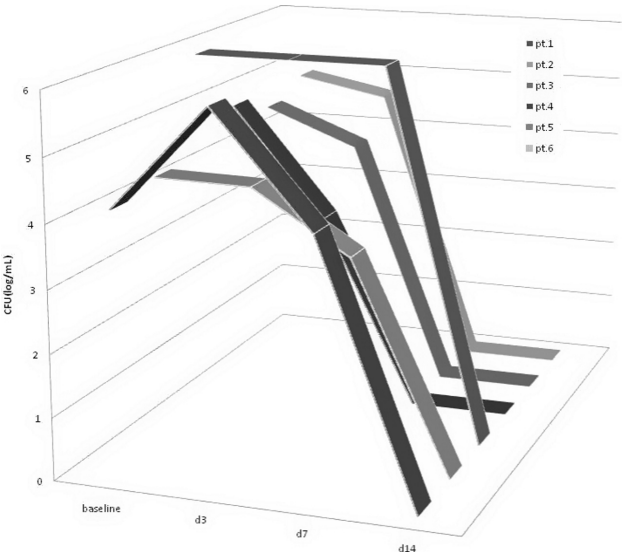

Figure 1. Reduction in CFU (log).

P1742 Efficacy and safety of liposomal amphotericin B in intensive care unit patients with confirmed invasive fungal infection: a retrospective, multi-centre study

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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 pathologies were medical (4.3%) and surgery pathology (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 creatinine 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 Micafungin versus liposomal amphotericin B for the treatment of serious *Candida* infections in intensive care unit and non-ICU patients: results of post-hoc analyses

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Objective: Assess efficacy and safety of micafungin (MICA) versus liposomal amphotericin B (L-AmB) for the treatment of invasive candidiasis (IC) or candidaemia 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.
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 = 206/263]; 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, multivariate 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

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Objectives: A number of agents are now available for empirical antifungal treatment (EAFT) 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 EAFT. Baselines patients characteristics at the time of EAFT 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 EAFT were recorded among 56 patients. All patients received high dose chemotherapy for either acute myeloid leukaemia (52%), acute lymphoid leukaemia (14%), lymphoma (13%) or other haematologic conditions (21%). Fifteen (27%) and 5 (9%) patients were allogeneic and autogenic haematopoietic 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 EAFT. 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 (dexoxycholate 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 EAFT. 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

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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

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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 (C/IC; P = 0.01). We assessed the economic impact of this therapy in hospital inpatients with C/IC.
Methods: Clinical and resource use data for 234 hospital inpatients with C/IC 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 ward (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 C/IC-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 (ANI 72.7% vs. FLU, 57.5%; 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 catheter status, total 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 C/IC, 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

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Objectives: Invasive pulmonary aspergillosis (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" (50, 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 antifungal drugs. 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 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

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Objective: 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 48 hrs 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.

Location	Voriconazole concentration (mg/L)							
Time since operation (hrs)	6	12	18	24	30	36	42	48
Patient 1								
Serum	0	0		0				0
Drain	306	251	179	169	92	46	43	51
Patient 2								
Serum	0.6	0.6		0.7				0.4
Drain	n/a	187	179	110	81	70	85	49

P1749 Voriconazole plasma levels monitoring in haemato-oncological patients

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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

intravenously. The total daily dose of voriconazole varied from 200 to 800 mg. The mean trough voriconazole plasma concentration after the standard daily dose 400 mg orally was 1.46 μg/ml (0.2–8.98 μg/ml). In 10.8% (47) of plasma samples from 27 (18.9%) patients the voriconazole plasma concentration was undetectable (<0.2 μg/ml). In 43.5% (n = 189) of samples from 70 (63%) patients after this standard dose (400 mg/day) the plasma concentration was under 1 μg/ml – below the level associated with a better response to therapy and better prognosis of invasive aspergillosis. The increase in daily voriconazole dose from 400 to 600 mg in 16 patients led to elevation of voriconazole trough plasma level above 1.0 μg/ml in 4 cases and above 0.5 μg/ml in 5 cases. In 7 patients the concentration did not changed or decreased.

Conclusion: Our results demonstrate that high percentage of patients did not achieved sufficient voriconazole plasma concentration after the standard daily dose of voriconazole. Monitoring of plasma levels enables an individual dosage adjustment and lead to improving response to antifungal treatment in patients in high risk of invasive fungal infection.

Daily doses of voriconazole	200 mg	400 mg	600 mg	800 mg
Number of samples	64	434	81	16
Number of patiens	18	111	20	2
Plasma concentration, μg/ml	0.75	1.46	1.44	2.24
Plasma concentration <0.2 μg/ml	8 (12.5%)	47 (10.8%)	6 (7.4%)	2 (12.5%)
Plasma concentration <1.0 μg/ml	51 (79.6%)	189 (43.5%)	41 (50.6%)	5 (31.3%)

P1750 Therapeutic drug monitoring of posaconazole in 54 adult patients

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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% (4/18) 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

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Invasive aspergillosis represents 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 mgx2/day and 200 mgx3/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) post Tx. 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 1084±310 mg/day, [733;1889], resulting in more than +35% of the recommended dose (p < 0001). 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 (voriconazole + 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 coprescription 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

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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 chemoradiotherpy following right extra-pleural pneumonectomy for malignant epitheloid 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]; prosthetic 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;prosthetic sheaths replacing the pericardium and diaphragm, under general anaesthesia and rib resection refashioned. 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.

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* Empyema is reported but would have not provided sustained local exposure in this unusual case. Video/picture to be presented.

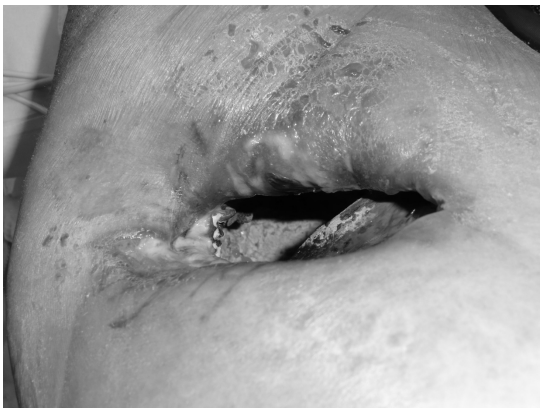

Figure 1.

Infections in the immunocompromised host

P1753 Febrile neutropenia in patients with haematological malignancies

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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 $\leqslant 500/mm^3$) due to chemotherapy. We recorded: fever >38ºC, 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 $\leqslant 100/mm^3$ vs 53.5% for ANC $> 100/mm^3$, $p < 0.01$). Bacteria were isolated in 559 of the febrile cases (62%). The ratio of Gram(+) bacteraemia to Gram(−) was 10 to 6. 30% of Enterococci were resistant to glycopeptides. The percentage of resistance of *Pseudomonas* sp. in carbapenems was also high (50%). Resistance of Enterobacteriacae to carbapemens 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 $\leqslant 100/mm^3$. 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 malignances. Preliminary report of a Spanish multicentre study

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Background: The aim of this study were to asses the principal "microbiological" features and clinical presentation of the patients with a haematological malignance (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 origins 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* peumoniae (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 admisión 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* peumoniae 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

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Objectives: To study and compare some characteristics of infections associated with central venous catheters in patients hospitalised in 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 semi-quantitative Maki's method and the quantitative Cleri's method. Blood culture samples were taken from a peripheral vein at the time of removing the catheter and incubated 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

was v. subclavia in 40/82–58/68 and v. femoralis in 42/82–10/68 cases respectively. The main duration of catheterisation was 8 days in ICU and 19 days in OUs. The most common microorganisms in ICU from 61/82 positive catheter tips were Gram negative rods: *A. baumannii* (21/61), *K. pneumoniae* (7/61), *P. aeruginosa* (7/61), *E. cloacae* (3/61), and *E. coli* (2/61) followed by Coagulase-negative staphylococci (18/61) and *Candida* sp (3/61). From 68 catheter tips sent from OUs, 25 had positive culture and in most of cases were isolated CNS (18/25) followed by *P. aeruginosa* (4/25) and *Candida* sp (3/25). As catheter related bacteraemias were considered in 25 cases, 20 in ICU and 5 in OUs, the causative microorganisms in ICU were Gram negative rods (15/25) while in oncology units were CNS (4/5).

Conclusion: The frequency of CRB in ICU (24.4%) is significantly higher than in oncology patients (7.3%). They developed earlier and were caused by Gram negative rods. Because the probable way to development of CRB is the skin of patients, the preventive measures should be focused on better aseptic techniques and training of the medical staff.

P1756 Sepsis in the elderly: different?

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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).

Variable	Control	Test
DM	32.3% (n = 21)	44.6% (n = 33)
HTN	38.5% (n = 25)	52.8% (n = 39)
CAD	27.7% (n = 18)	44.6% (n = 33)
MV	49.2% (n = 32)	43.2% (n = 32)
RRT	56.9% (n = 37)	37.9% (n = 28)
LOS (days)	8.7+3.9	7.8+3.8
SOFA scores	13.5+3.9	15.3+3.9
Culture positive	40% (52/130)	44.6% (66/148)

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) for control group and MSSA (n = 6), MRSA (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.

P1757 The production of ESBLs does not affect the early and late clinical outcome of *E. coli* or *K. pneumoniae* bacteraemia in febrile neutropenia

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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 bacteraemia 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* bacteraemia 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* bacteraemia 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 bacteraemia 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 bacteraemia 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.

P1758 Taxonomic structure of anaerobic bacteria in cancer patients in a Russian cancer research centre

I. Shilnikova, N. Dmitrieva (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 2 h of collection and special anaerobic transport systems were not used. Cultivation methods included enrichment media and GasPak jar. Isolates were identifined 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.

P1759 Infections caused by Gram-positive anaerobic cocci in cancer patients

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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 2 h 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 37C for 48–96 h 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%), *Peptoniphilus 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 doxicycline. Over 50% *F. magna* strains were resistant to clindamycin, cefotaxime and ceftriaxone; 30–40% – to fluoroquinolones, including moxifloxacin, and 60–70% – to azithromycin and erytromycin. In *P. anaerobius* strains only resistance to fluoroquinolones (33%) was found. *P. asaccharolyticus* strains demonstrated intermediate resistance to azithromycin (22%) and erytromycin (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)

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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. However, the true spectrum of infections in patients receiving this TKI remains unknown. To this effect, we evaluated all infections during dasatinib therapy in patients at our tertiary care cancer centre.

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)
CoNS CrBSI	1 (2)
Enterococcus CrBSI	2 (5)
MRSA CrBSI	2 (5)
MRSA otitis extema	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 krusei CrBSI	1 (2)

*2 cellulites/1 perirectal abscess; **1 neutropenic enterocolitis.
CrBSI: catheter-related bloodstream infection; CoNS: coagulase-negative staphylococci; MRSA: methicillin resistant *Staphylococcus aureus*.

P1761 Biomarkers of infection and septic shock in neutropenic patients

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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 >10 ng/ml), CRP (N < 6 mg/l), serum bicarbonate (N: 24–26 mmol/L), serum lactate (N < 2.2 mmol/L) and phosphataemia (N:0.8–1.4 mmol/L).

50 neutropenic febrile episodes were noted among 27 patients with acute leukaemia. All patients presented neutropenia (ANC $< 0.5 \times 10^9$/L) lasting for more than 7 days. They were on oral anti-bacterial (polymixin B, gentamicin) and anti-fungal (fungizone) prophylaxis. The first neutropenic febrile episode was treated with Piperacillin-Tazobactam and Polymixin B IV, if the patient remain febrile at 48 hour from the start of this first line the fungizone iv is added. Imipenem 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 < 0.5 ng/ml was noted in 18 (36%) febrile episodes, whearas high level (>10 ng/ml) are noted in 11 episodes. Median level of each biomarker was: CRP 88.8 mg/L (range, 2.2–183 mg/L), Serum lactate 2.3 mmol/L (range, 0.8–3.3 mmol/L), Serum bicarbonate 24.3 mmol/L (range, 9.4–32.1 mmol/L), and phosphataemia 1.08 mmol/L (range, 9.4–32.1 mmol/L).

PCT > 0.5 ng/ml ($p = 0.004$; OR = 6.6) and CRP > 100 mg/L ($p = 0.008$; OR = 6.1) were correlated with microbiologically and/or clinically documented infection. CRP > 100 mg/L correlates with Gram– infection ($p = 0.045$). PCT > 10 ng/ml ($p = 0.017$; OR 10.5) and serum lactate >3 mmol ($p = 0.04$; 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)

[1] Clin Infect Dis 2002;34:730–751.
[2] Crit Care Med 1992;20:864–874.

P1762 Can multiplex PCR (SeptiFast) detect DNAemia before occurrence of sepsis in neutropenic patients?

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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 haematopoietic 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 >72 h.

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 diseasesincluded acute myeloic 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

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Background: Febrile neutropenia [FN] is a common emergency in Haemato-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

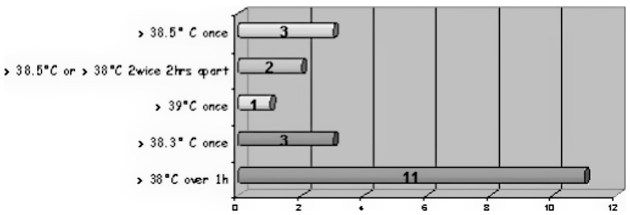

Figure: Definition of FEVER in the policy.

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 guidelance 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. Liposomal amphotericin and caspofungin were common options. Itraconazole [prophylaxis] in leukaemia/lymphoma patients [>90]. Piperacillin-tazobactam/gentamicin or carbapenem used 1st line

in >85%. Teicoplanin [57% /single daily dosing] was preferred. Patient information card [PIC] detailing chemo regimes/dates offered by 29%; Contact number for direct admission to haematology offered 71%. Variation in suggested use of antifungals noted between the standards.
Recommendations: There is an evident need for a national [UK] guideline on diagnosis and management of FN. Appropriate and timely specimen collection could be optimised. PIC should be provided by all centres. Choice, appropriateness and timing of antifungal agents requires definite clarity in hospital FN policies. Smaller units with limited experience could avoid addressing risk stratification in hospital FN policy and a potential risk of under-treating a high-risk patient with FN leaving this to specialist centres.

P1764 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

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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 standart empirical regimen of intravenous ceftazidime 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, diarrhoea, 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.

P1765 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

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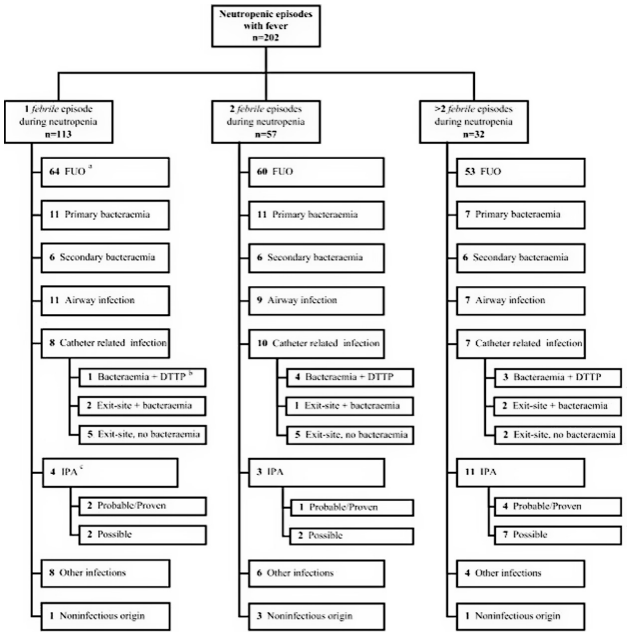
Objectives: Guidelines advocate at least 7 days of broad-spectrum antimicrobial therapy for unexplained fever during neutropenia. However, effective antimicrobial prophylaxis reduces the incidence of Gram-negative infections, which may allow shorter treatment. This study evaluates the safety of discontinuing 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 $\geqslant$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 $\geqslant$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.



Cause of fever in 202 neutropenic episodes (137 patients). Febrile episodes caused by the same aetiology during a single period of neutropenia were counted only once, therefore adding up totals may give slightly different numbers compared to results when multiplying the number of neutropenic periods with the number of febrile episodes. [a] FUO, fever of unknown origin. [b] DTTP, differential time to positivity. [c] IPA, invasive pulmonary aspergillosis, classified according to updated EORTC-MSG criteria.

P1766 Antiviral prophylaxis in haematological patients: systematic review and meta-analysis

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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-seropositive (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

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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, 76.5%), 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 mycofenolate 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.

Infections in transplant recipients

P1768 Detection of cytomegalovirus resistance to antivirals in paediatric haematopoietic stem cell transplant recipients: study in a paediatric cohort in the Czech Republic

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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 (alloHSCT) 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 alloHSCT 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 foscarnet 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 polymorphisms in UL54 were detected in 16 patients (8%). Four novel changes of unknown phenotype in UL97 and 6 in UL54 were detected. The detection of GCV resistance of CMV led to the switch of the treatment in 4 patients, with success in 3.
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.
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P1769 Treatment failure and antiviral resistance in cytomegalovirus infections in stem cell transplant recipients

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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 pre-emptive 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 A594V 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.

P1770 Clinical applicability of a diagnostic DNA-microarray for the simultaneous detection of herpesviruses and adenovirus co-infections in patients undergoing allogeneic stem cell transplantation

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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 (VINAray) 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 herpes-*simplex*-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 devided 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 (35%, 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.

P1771 Incidence, timing and aetiology of bloodstream infections following orthotopic liver transplantation or haematopoietic stem cell transplantation – a single-centre experience

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Bloodstream infections (BSI) are major complications of orthotopic liver transplantation (OLT) and haematopoeitic stem cell transplantation (HSCT). Knowing characteristical 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.

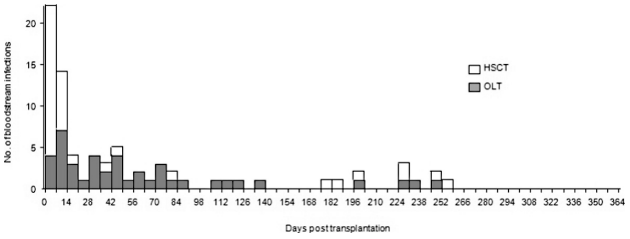

Figure 1. Incidence of bloodstream infections in patients after haematopoietic stem cell transplantation (HSCT) or orthotopic liver transplantation (OLT).

P1772 Outbreak of *Ralstonia pickettii* bacteraemia in patients with haematological malignancies and haematopoietic stem cell transplant recipients

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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). Of them, 9 were cared for in the HSCT Unit, both in inpatient and outpatient facilities, and 1 was admitted for chemotherapy to another haematology unit in the same hospital.
Results: Clinical and microbiological features of the patients are shown in Table 1.

Table 1. Clinical and microbiological features of patients with *Ralstonia pickettii* bacteraemia

						No. of positive blood cultures								
Pts	Gender	Age	Underlying disease	Date of HSCT	Date of bacteraemia	Total	CVC	Peripheral vein	CRP	Fever	Sepsis	CVC removed	Days to blood cultures negative	Alive
1. PC	F	55	NHL	–	03/06/08	1	1	0	ND	–	–	+	1	–
2. DO	M	24	AML	–	17/06/08	3	2	1	180	+	+	–	2	+
3. SL	M	40	ALL	–	26/06/08	2	0	2	51	+	+	+	ND	+
4. CL	F	59	NHL	23/07/08	22/07/08	2	1	1	Neg	–	–	–	1	+
5. CR	M	54	AML	23/03/08	08/08/08	1	0	1	Neg	–	–	–	ND	+
6. SG	M	36	ALL	21/05/08	08/08/08	6	4	2	Neg	–	–	+	18	+
7. TV	M	63	MF	24/01/08	08/08/08	10	8	2	25	+	+	+	12	+
8. CV	M	53	MDS	26/09/06	10/08/08	1	1	0	179	–	–	+	1	+
9. RD	M	55	NHL	–	13/08/08	1	1	0	Neg	–	–	–	ND	+
10. CG*	M	36	HD	15/06/07	19/08/08	6	5	1	58	+	+	+	9	+

*Two separate episodes of *R. pickettii* bacteraemia, the first one occurred on 16th June 2008, and was treated in a different hospital.
ALL, acute lymphatic leukaemia; AML, acute myeloid leukaemia; CRP, C-reactive protein; HD, Hodgkin disease; MDS, myelodysplastic syndrome; MF, myelofibrosis; ND, no data; Neg, negative, NHL, non-Hodgkin lymphoma.

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 aminoglycosides, fluoroquinolones, 3rd and 4th generation cephalosporins, piperacillin/tazobactam and carbapenems, and resistant to aztreonam.
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 cephalosporins or meropenem. The symptomatic patients improved and blood cultures became negative after a median of 2 days (range: 1–18). Six patients had the central venous catheter removed and 1 tip culture was positive for *R. pickettii*, 1 for *K. pneumoniae* and 3 were negative. No patient died due to *R. pickettii* BSI; 1 patient died of other causes (cardiomyopathy).
Epidemiological and microbiological investigations were undertaken and environmental samples, together with samples of several potential contaminated substrates (chlorhexidine, sterile saline and water, glucose and heparin solutions, hand-washing antiseptic soap) were cultured. Additionally, all the existing hygiene and infection control procedures were reviewed and actively monitored. All the cultures resulted negative for *R. pickettii*.
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.

P1773 Tailor-made therapy to prevent postoperative sepsis in living donor liver transplantation

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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 immunological status: 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.

P1774 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

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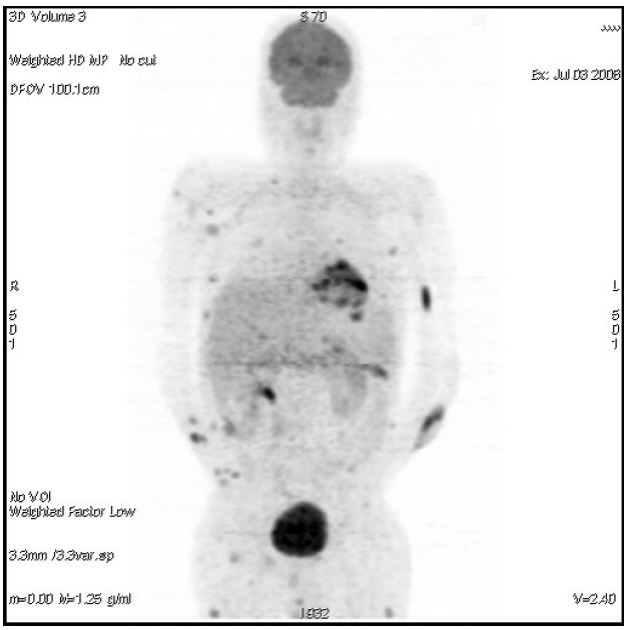
Invasive aspergillosis often presents as a respiratory tract infection in transplant patients, but after haematogenous disssemination virtually any other organ may be involved. Metastatic cutaneous aspergillosis usually presents as necrotising cutaneous papules or ulcerating skin lesions due to embolisation and skin infarcts. 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

remained very prostrated and 1 month later painless subcutaneous nodules were noted. An excised nodule revelead infiltration of the subcutaneous tissue by hyphae and cultures grew *Aspergillus fumigatus*. He was apyrexial, non-neutropenic and had no respiratory tract symptoms but a computorised tomography scan showed confluent lung opacities and serum galactomannan was strongly positive. A PET scan revealed numerous lesions involving the limbs, chest wall and lungs. Voriconazol was replaced by Ambisome® and caspofungin in combination, followed by posaconazol and high dose Ambisome® to no avail since the patient died 4 months later.

Disseminated aspergillosis presenting with cutaneous nodules and intact overlying skin is extremely unusual. The PET scan allowed an evaluation of the metastatic infection supporting the interest of this technique in assessing the extent of the disease. Profound immunosuppression and steroid therapy allowed for invasive aspergillosis despite prolonged theoretically protective prophylaxis and adequate neutrophils. The best management of breakthrough invasive aspergillosis in the context of mould-active azole prophylaxis is not known as there are no clinical studies to support an adequate strategy. It should be individualised on the basis of clinical criteria as well as consideration of other antifungal drug classes. Treatment with several antifungal combinations proved ineffective in controlling the infection, emphasizing the need for an increased awareness of invasive fungal infections, even when mould-active antifungal prophylaxis is given.



P1775 A decision support system for diagnosis and treatment of gastrointestinal tract infections in solid-organ transplant recipients

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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 were able to 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.

P1776 Amphotericin B as antifungal prophylaxis in liver transplantation

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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 analyse 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 15year 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 15year 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.

P1777 Long-term evaluation of pre-emptive treatment after solid organ transplant in patients at high risk for cytomegalovirus infection

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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 antigenaemia. 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 antigenaemia. Moreover, within the same patients positive antigenaemia 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 occured 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.

P1778 Current status of CMV reactivations in adult liver transplant patients monitored by frequent quantitative PCR testing

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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 seroposive 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-DNAemia 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.
Conclusions: 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.

P1779 Valganciclovir prophylaxis for CMV infection in thoracic transplant patients

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Cytomegalovirus (CMV) infection is a very common opportunistic infection after solid organ transplantation. Intravenous Valganciclovir (vGCV), an haematotoxic 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 antigenaemia (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±67 days in case of HT versus 270±85 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 neutropenia 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%.
Conclusion: 900 mg vGCV 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 a 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

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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 occcurring 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

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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 transplantation referred to our Infectious Diseases Department. On the other hand, we noticed that presence of antitoxoplasma IgM in donors correlates with an 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, Toxok IgA (Diasorin 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(Clonit 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 bacteraemia: pooled analysis from 8 phase 3 clinical trials

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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 intraabdominal 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

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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.

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 defervescence 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 conditions 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 groups, respectively. No statistical differences in the duration of the illness were found before therapy among the 4 treatment groups.

The mean time of 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 as compared with patients given cotrimoxazole, 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 (10.05days), 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

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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-trimoxasole – 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 tromitamin.

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; with >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 400 mg twice a day for a 3 days, patients of the Group 2 were treated by one dose of fosfomycin tromitamine 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 Groupe 1 and 2 (Table 1). Clinical improvement, cure and failure rate at V2 were 98.2%, 68.5%, 1.9% for Groupe 1 and 76%, 98%, 2.0% for Group 2. Eradication and persistence rate at V2 were 100% and 0% for Groupe 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

	Group 1	Group 2	P value
Number of patients included	55	53	
Age, M±m	35.0±11.0	31.4±8.4	>0.05
Duration of UTI, days, M±m	2.1±0.8	2.0±0.8	>0.05
Isolated recovered before treatment, n/N(%)			
E. coli	45/53 (84.9%)	39/49 (79.6%)	>0.05
E. faecalis	3/53 (5.7%)	3/49 (6.1%)	>0.05
S. saprophyticus	2/53 (3.8%)	2/49 (6.1%)	>0.05
Staphylococcus spp.	1/53 (1.9%)	1/49 (2.0%)	>0.05
Other	2/53 (3.8%)	3/49 (6.0%)	>0.05
Microbiological efficacy, n/N(%)			
Eradication rate (on V2)	54/54 (100%)	46/48 (95.8%)	>0.05
Persistence (on V2)	0/54 (0%)	2/48 (4.3%)	>0.05
Reinfection (on V3 or V4)	1/21 (4.8%)	0/15 (0%)	>0.05
Relapse (on V3 or V4)	1/21 (4.8%)	0/15 (0%)	>0.05
Clinical efficacy, n/N(%)			
Cure (on V2)	37/54 (68.5%)	38/50 (76%)	>0.05
Improvement (on V2)	53/54 (98.2%)	49/50 (98.0%)	>0.05
Failure (on V2)	1/54 (1.9%)	1/50 (2.0%)	>0.05
Relapse (on V3 or V4)	1/37 (2.7%)	0/20 (0%)	>0.05
Adverse events, n/N(%)	1/54 (1.9%)	0/53 (0%)	>0.05

P1785 Efficacy of IV/oral moxifloxacin in the treatment of complicated skin and skin-structure infections: results of the RELIEF study

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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 4.0/0.5 g 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

Populations	MXF n/N (%)	PIP/TAZ-AMC n/N (%)	95% CI
Clinical response			
Per-protocol	322/363 (88.7)	275/307 (89.6)	−5.2, 4.0
MBV	235/270 (87.0)	215/243 (88.5)	−7.4, 3.5
ITT	353/426 (82.9)	305/377 (80.9)	−3.1, 7.0
ITT with organisms	256/313 (81.8)	234/290 (80.7)	−5.2, 6.6
Bacteriological response			
MBV	224/270 (83.0)	210/243 (86.4)	−10.2, 1.7
ITT with organisms	244/313 (78.0)	227/290 (78.3)	−7.5, 5.0

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 bacteriological 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%, P = 0.60, respectively).
Conclusion: 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.

P1786 Efficacy of IV/oral moxifloxacin and IV piperacillin/tazobactam followed by oral amoxicillin-clavulanic acid in the treatment of major abscess: results of the RELIEF study

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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 cSSSI 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 incision and drainage, were stratified according to infection severity and randomised to IV/PO MXF 400 mg qd or IV piperacillin/tazobactam 4.0/0.5 g tds followed by PO amoxicillin/clavulanic acid (PIP/TAZ-AMC) 875/125 mg bd, for 7–21 days. The diagnosis of major abscess was documented by repeated pictures and confirmed by an independent data review committee. The primary efficacy variable was clinical response 14–28 days after completion of therapy (test-of-cure, TOC). Non-inferiority of MXF to PIP/TAZ-AMC was demonstrated if the lower limit of the 95% CI was >−10%.
Results: A total of 354 patients (MXF=184, PIP/TAZ-AMC=170) with an abscess were randomised and 352 (MXF=183, PIP/TAZ-AMC=169) were valid for ITT/safety analyses. In the PP population (MXF=167, PIP/TAZ-AMC=153), 94 (56.3%) MXF- and 93 (60.8%) PIP/TAZ-AMC-treated patients had involvement of fascia, muscle or deeper tissues; fat was the deepest tissue involved in the remaining patients (MXF: 73, 43.7%; PIP/TAZ-AMC: 60, 39.2%). Among PP patients, 111 (34.7%) required local or extensive debridement in addition to surgical drainage. Mean (SD) lesion area was (MXF) 111 (141) cm2 and (PIP/TAZ-AMC) 110 (161) cm2. Abscesses of the rectal area (64.2%) 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). Bacteriological 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.

Table: Clinical and bacteriological responses at TOC

	MXF n/N (%)	PIP/TAZ n/N (%)	95% CI
Clinical response			
Per-protocol	160/167 (95.8)	147/153 (96.1)	−4.2, 4.5
MBV	119/125 (95.2)	113/117 (96.6)	−5.5, 4.3
ITT	163/183 (89.1)	151/169 (89.3)	−5.6, 7.2
ITT with organisms	122/135 (90.4)	114/125 (91.2)	−5.6, 8.1
Bacteriological response			
MBV	117/125 (93.6)	113/117 (96.6)	−7.5, 3.1
ITT with organisms	119/135 (88.1)	114/125 (91.2)	−8.0, 6.3
Bacteriological response by key organism			
Staphylococcus aureus			
Methicillin-susceptible	65/69 (94.2)	66/67 (98.5)	0.366†
Methicillin-resistant	4/4 (100)	2/2 (100)	–
Escherichia coli			
non-ESBL	28/31 (90.3)	22/23 (95.7)	0.628†
ESBL	3/3 (100)	0	–
Enterococcus faecalis	13/14 (92.9)	9/10 (90.0)	1.000†
Streptococcus pyogenes	23/23 (100)	15/17 (88.2)	0.174†
Bacteroides fragilis	16/20 (80.0)	9/11 (81.8)	1.000†

†Fisher's exact test. MBV: microbiologically valid.

P1787 Single-dose antibiotic prophylaxis for urinary catheter removal does not reduce the risk for urinary tract infection in surgical patients: a randomised double-blind placebo-controlled trial

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Objectives: We conducted a double-blind, placebo-controlled trial to assess the efficacy of singledose therapy of trimethoprim-sulfamethoxazole (co-trimoxazol) or ciprofloxacin versus placebo therapy in surgical patients with scheduled bladder drainage for 3–14 days.
Methods: Patients were randomly assigned to receive either placebo, a single-dose of co-trimoxazol (960 mg) or a single-dose of ciprofloxacin (500 mg). Primary outcome measures were significant bacteriuria and UTI. Urine cultures were obtained directly after urinary catheter removal and 12–14 days after urinary catheter removal, in combination with a questionnaire for UTI symptoms. This trial was registered with ClinicalTrials.gov, number NCT00126698.
Results: Thirty-five percent of analysed placebo patients (n = 44) had significant bacteriuria directly following catheter removal compared with 9% of patients receiving ciprofloxacin prophylaxis (n = 34) (p = 0.01) and 27% patients receiving co-trimoxazol prophylaxis (n = 37) (p 0.43). Two weeks after catheter removal there was no significant difference in bacteriuria between the placebo group and the two prophylaxis groups. UTI was found in 2.9% of the placebo group, versus 3.2% in the ciprofloxacin group (p = 0.93) and 0% in the cotrimoxazol group (p = 0.40).
Conclusion: Ciprofloxacin prophylaxis is effective in resolving asymptomatic bacteriuria directly following catheter removal; however, overall there was no significant benefit of prophylaxis with respect to reduction of bacteriuria or rate of UTI two weeks after catheter removal. Because of the lack of evidence for short- or long-term adverse outcomes, treatment of asymptomatic bacteriuria has not been recommended. We conclude that surgical patients do not benefit from antibiotic prophylaxis for urinary catheter removal.

P1788 Catheter exit site infections in patients undergoing continuous ambulatory peritoneal dialysis

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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. lwoffii*, *P. mirabilis*, *E. cloacae*, *S. marcescens*, *K. pneumoniae*, *E. coli*, *Morganella morganii*, *Moraxella*, *P. putida*, *Sphingomonas paucimobilis*, *Achromobacter 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 I was susceptible only to glycopeptides and rifampicin. All Gram-negative bacteria were multisusceptible 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 empiric 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).

P1789 Effect of intermittent moxifloxacin therapy on the microbiology of sputum cultures from patients with chronic obstructive pulmonary disease (the PULSE study)

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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).

Isolate	Patients with isolate N at EOT/N at randomisation (%)			Median net change in MIC value (mg/L) (from randomisation to EOT)	
	MXF	Placebo	P value[a]	MXF	Placebo
H. influenzae	4/24 (17)	28/36 (78)	<0.001	None	None (−0.06)
H. parainfluenzae	9/27 (33)	14/22 (64)	0.047	None	None (−0.03)
S. pneumoniae	6/15 (40)	12/16 (75)	0.073	None	None
S. aureus	5/8 (63)	2/11 (18)	0.074	None (−0.02)	None (−0.02)
P. aeruginosa	7/9 (78)	11/8 (138)	1.000	None (−0.5)	1.625

[a] Fisher's exact test.

MIC values to MXF remained stable for most bacterial species isolated from sputum during the study. For *S. aureus* and *P. aeruginosa* there were transient increases in MIC in some isolates during the study, but these appeared to be independent of treatment arm or timing of treatment.

Conclusion: The higher clinical efficacy of intermittent MXF therapy in the reduction of exacerbations in COPD correlates with a higher eradication of primary pathogens compared to placebo treatment. There was no consistent MXF-related increase in MIC during the 48-week PULSE study or the 24-week follow-up period. Therefore chronic intermittent therapy with MXF was effective in reducing bacterial burden without an associated increase in the emergence of resistant bacteria during the treatment period.

P1790 Safety and clinical outcomes of high-dose daptomycin (>6 mg/kg)

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Objectives: Daptomycin, a bactericidal, concentration-dependent antibiotic, has been administered safely to healthy volunteers in doses as high as 12 mg/kg for 14 days and to patients (pts) with skin and soft-tissue infections (SSTI) at 10 mg/kg for 4 days. The objective of this study was to describe the use of daptomycin at doses >6 mg/kg for extended durations.

Methods: Data were collected in CORE (2005 to 2007 program years), a retrospective, observational registry of pt experience in the US. All pts who received daptomycin >6 mg/kg were evaluated for safety through 30 days after completing daptomycin and efficacy at end of daptomycin therapy. Clinical outcomes (cure, improved, failure, and nonevaluable) were assessed using protocol-defined criteria. Success was defined as cure or improved. Nonevaluable pts were excluded from the efficacy analysis.

Results: Two hundred thirty-one of 3617 (6%) received daptomycin >6 mg/kg; 94 of 231 (41%) patients received doses ⩾8 mg/kg. Overall, the infection types with the highest use of doses >6 mg/kg were infective endocarditis (IE, 11%) and bacteraemia (BAC, 8%). The percentage with BAC, SSTI and IE were: 75/231, 32%; 64/231, 28%; and 17/231, 7%, respectively. The most common causative pathogens were *S. aureus* (83/231, 36%; methicillin-resistant 72%) and *Enterococcus* sp. (66/231, 29%; vancomycin-resistant 59%). Fifty percent were female, 39% were ⩾66 yrs old, 24% initial creatinine clearance <30 mL/min, 12% on dialysis, and 27% received daptomycin in an intensive care unit. Diabetes was present in 28% and 18% had a history of cancer. The median (min, max) daptomycin duration of therapy was 10 days (1, 90; 71% ⩾5 days). Twelve (5%) of the 231 pts experienced 1 or more adverse events (AE) possibly related to daptomycin. Fifteen (6.5%) of the 231 pts discontinued daptomycin due to AE; however, it was due to a possibly related AE in only 6 (2.6%). An elevated creatine phosphokinase (CPK) was reported in 4 (1.7%) patients. One hundred seventy-three (75%) patients were considered evaluable for efficacy. The overall clinical

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.

P1791 Safety of daptomycin in the treatment of osteomyelitis: results from a registry

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Objectives: 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 cure, 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 per kg or greater may be associated with greater clinical improvement. Ideally, these results should be confirmed via a prospective clinical trial.

P1792 CANVAS-2: randomised, double-blinded, phase 3 study (p903–07) of the efficacy and safety of ceftaroline versus vancomycin plus aztreonam in complicated skin and skin-structure infections

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Objectives: 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: Adult patients with cSSSI requiring IV therapy received CPT 600 mg q12 h or V 1 g plus A 1 g q12 h 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, −4.4 to 4.5) 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 diarrhoea (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.

P1793 Clinical cures with oral linezolid versus intravenous vancomycin in propensity score-matched subjects with complicated skin and soft-tissue infections proven to be due to methicillin-resistant *Staphylococcus aureus*

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Objectives: Linezolid (LIN), an oxazolidinone antibiotic, is 100% bioavailable in oral (PO) and intravenous (IV) formulations. Clinical outcomes of treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) complicated skin and soft tissue infections (cSSTI) with PO LIN vs IV vancomycin (VAN) have not been well described. Data from a previously reported randomised, open-label study of LIN vs VAN in proven MRSA cSSTI allow post hoc comparison of outcomes in PO LIN- vs matched IV VAN-treated subjects.
Methods: Subjects ⩾18 y were randomised to LIN (600 mg IV or PO q12 h) or VAN (15 mg/kg IV q12 h, adjusted for CLcr) for 7–14 d. Subjects who met key protocol criteria were included. Characteristics differentiating PO LIN (n = 95) from IV LIN (n = 134) subjects were identified by logistic regression. PO LIN and IV VAN subjects were retrospectively matched based on region, age, presence of diabetes, hospitalisation, and signs/symptoms using propensity score (PS) methodology. Clinical outcomes in PO LIN and IV VAN subgroups were compared at end of study (7–10 d after last dose).
Results: Of 95 PO LIN and 210 IV VAN subjects evaluated, 82 per group had comparable PS. Mean age was 50 vs 48 y; 82.9% vs 84.1% of subjects were hospitalised, and 26.8% vs 25.6% had diabetes (PO LIN vs IV VAN, respectively). Number of signs/symptoms was similar in both groups. Clinical cure was achieved in 96.3% (78/81) of PO LIN subjects vs 87.8% (72/82) of IV VAN subjects (Chi square test, p = 0.045; Fisher exact test, p = 0.079). 1 PO LIN subject had unknown response. Median length of hospital stay was similar for both groups (3.5 d PO LIN; 4 d IV VAN). Overall adverse event (AE) rates were similar in each group (58.5% PO LIN [48/82] and 59.8% VAN [49/82]), with gastrointestinal symptoms more prevalent in PO LIN and allergic symptoms more prevalent in IV VAN.
Conclusions: PO LIN showed comparable clinical cure rates to those achieved with IV VAN in PS-matched subjects with proven MRSA cSSTI. Overall AEs in both groups were similar, and consistent with the known safety profile of each drug.

P1794 Results of a phase 2 study comparing two doses of delafloxacin to tigecycline in adults with complicated skin and skin-structure infections

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Objectives: Delafloxacin (DFX) is an investigational fluoroquinolone active against a variety of Gram-positive bacteria, including methicillin- and quinolone-resistant strains of *Staphylococcus aureus* (MRSA,

QRSA). Two doses of DFX were compared to a standard dose of tigecycline (TIG) in patients with complicated skin and skin structure infections (cSSSI).
Methods: A multicentre, randomised, double-blind trial enrolled adults with the following: wound infections following surgery, trauma, burns or animal/insect bites; abscesses; or cellulitis. Patients were randomised 1:1:1 to receive either DFX 300 mg IV BID, DFX 450 mg IV BID, or TIG, 100 mg IV×1, followed by 50 mg IV BID; randomisation was stratified by infection type. Duration of therapy was 5 to 14 days. The primary efficacy analysis, performed on the clinically evaluable (CE) population at the Test-of-Cure (TOC) visit (14 to 21 days after the final dose of study drug), compared the clinical response rates in the DFX and TIG arms. Clinical response rates in the two DFX treatment arms were also compared. The Fisher exact test was used for the comparisons.
Results: Of the 150 patients randomised, 68% were male; the mean age was 40±14.5 yrs. Thirty-six percent had cellulitis, 33% had abscesses and 31% had wound infections; 111 (74%) patients had pathogens identified at baseline. *S. aureus* (95) was the most frequent isolate; 72% (68/95) were MRSA (MIC90 values, in mcg/mL, were as follows: DFX = 0.06, TIG = 0.12, ciprofloxacin = 16, levofloxacin = 4, linezolid = 1). Overall, the most frequent adverse events were nausea, vomiting and diarrhoea; the 300 mg BID DFX arm was the best tolerated of the regimens.
Conclusions: DFX, dosed at either 300 mg BID or 450 mg BID, was as effective as TIG 50 mg BID when used to treat adults with a variety of cSSSIs, including those caused by MRSA and QRSA.

	Percent Clinical Cure at TOC		
Population	DFX 300 BID	DFX 450 BID	TIG 50 BID
Clinically evaluable	97.2% p = 0.3498* p = 0.6170^{+}	92.5% p = 1.0000*	91.20%
Modified Intent-to-Treat	89.8% p = 0.3881* p = 1.0000^{+}	90.2% p = 0.2632*	82.00%

*p value for comparison between DFX and TIG group.
$^{+}$p value for comparison between DFX groups.

P1795 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

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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 (75 mg/m^2 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

P1796 Comparative antimicrobial susceptibility of biofilm versus planktonic forms of *Salmonella* spp. from children with gastroenteritis

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Biofilms are involved in a variety of bacterial and fungal infections. Biofilm bacteria produce an extracellular polymeric substance (ESP), which protects them against antimicrobial agents, thus leading to a 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%, ampicilln 12.8% and 84.4%, coamoxiclav 0% and 51.4%, cotrimoxazole 0.9% and 63.3%, cefuroxime 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 cotrimoxazole. 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.

P1797 Evaluation of aggregative adherence and biofilm formation in β-lactamase producing *Proteus mirabilis* isolates from different clinical settings in Italy

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Objectives: *P. mirabilis* is the second most relevant uropathogen and urinary tract infections (UTIs) caused by TEM-92 ESBL and CMY-16 CBL producers are increasing in Italian settings. In catheterised patients UTI may become chronic, with a microbial persistence maybe due to both antibiotic resistance and adhesion. 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 mrpA gene encoding for the major fimbrial subunit of MR/P fimbriae was investigated by PCR. 4 selected strains were also tested for adherence to LLC-MK2 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. mrpA gene was detected in all strains; the 4 strains studied for adherence to LLC-MK, 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 wich 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. Altough the results didn't fully support a direct correletion between BL production, biofilm and persistence, both these mechanisms contribuite to UTIs chronicness

P1798 Bioelectric effect decreases alginate production in *Pseudomonas aeruginosa* biofilms

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Objectives: Low electrical currents are able to increase biocides activity against microorganisms 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 ($4\,mg\,l^{-1}$) and gentamicin ($10\,mg\,l^{-1}$) 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 $18{,}000\times g$ at 4ºC. The clear supernatant was heated for 30 min at 80ºC to kill viable bacteria and passed through a 0.45μ 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, $P \leqslant 0.05$.
Results: The antibiotics alone reduced the biofilm population and in the presence of bioelectric effect the viable population was further reduced by gentamicin and especially by amikacin due to 47% and 60% reduction in alginate production, respectively.

Alginate production in *P. aeruginosa* strain 8821 after treatment with 0 and $9\,mA/cm^2$ electrical current in combination with antibiotics.

Electric current	Antibiotics	Time		
		8	16	24
$9\,mA/mm^2$	Amikacin	1.44	1.11	0.63
DC	Gentamicin	2.27	1.64	0.91
	No antibiotic	2.54	2.86	3.13
$0\,mA/mm^2$	Amikacin	2.15	1.92	1.10
DC	Gentamicin	2.33	2.10	1.94
Control	No antibiotic	2.79	3.22	3.48

Conclusion: The bioelectric effect reduced the alginate production in *P. aeruginosa* and enhanced the penetration of effective antibiotics into the biofilms.

P1799 Lack of differences in mutation frequency rates between cystic fibrosis *Pseudomonas aeruginosa* isolates in planktonic and biofilm type of growth

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Objective: To compare the mutation frequency of *Pseudomonas aeruginosa* isolates from cystic fibrosis (CF) patients using both planktonic and biofilm type of growth.
Methods: A total of 42 *P. aeruginosa* isolates recovered from sputum samples of 10 CF patients attending our hospital during 2004–2008 were studied. Genetic relatedness was performed by PFGE-XbaI and antibiotic susceptibility of planktonic cultures was determined by the standard CLSI agar dilution method. Biofilm formation was developed along 3 days on the surface of nitrocellulose filter disks inoculated with 100 μl of an overnight LB broth culture. Mutants were detected on rifampin-containing (300 μg/ml) LB agar plates from both types of cultures: 10 ml LB broth of the planktonic culture obtained after 18-h incubation under shaking, and the whole nitrocellulose biofilm-culture eluted with 0.5 ml of saline. To determine viable cells, different dilutions of the two-types of original inocula were plated onto BHI agar. Mutation frequencies were expressed as the ratio of no. mutants/no. viable cells. All experiments were performed in triplicate and results correspond to the mean value. The *P. aeruginosa* PAO1 (normomutator) and PAO1deltamutS (hypermutator) strains were included as controls.
Results: Eighteen PFGE patterns were found among the 42 isolates, being 3 pulsotypes present in two different patients. Antibiotic susceptibility tests demonstrated that although being multi-resistant, all isolates were susceptible to 100 μg of rifampin per ml. In general, a similar mutation frequency rate was detected when considering planktonic cultures (5×10^{-6} to 6.2×10^{-10}, mean: 4.8×10^{-7}) or when using biofilm growing cultures (3.3×10^{-6} to 3.3×10^{-9}, mean: 2.7×10^{-7}). However, in 28 strains the mutation frequency rate decreased (mean value, 4.6 times) in biofilm-growing cultures while in the other 14 strains it increased (mean value 4.5 times). For the PAO1deltamutS strain, mutation rate values were 1×10^{-6} to 8×10^{-7} in planktonic and biofilm type of growth, respectively, being of 2.5×10^{-8} to 7.9×10^{-9} for the PAO1 strain.
Conclusion: The mutation frequency rate to rifampin of CF-*P. aeruginosa* isolates growing in planktonic conditions were similar to that obtained in biofilm-growing conditions. This result indicates that other factors different that bacterial type of growth might influence in the increase of mutation frequency of *P. aeruginosa* in CF patients.

P1800 Mixed biofilms formed by *Haemophilus influenzae* and *Streptococcus pneumoniae* and expression of certain biofilm-related genes

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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 16 s 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 5.98 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.

P1801 In vitro interference of cefditoren on slime production by *Streptococcus pneumoniae*

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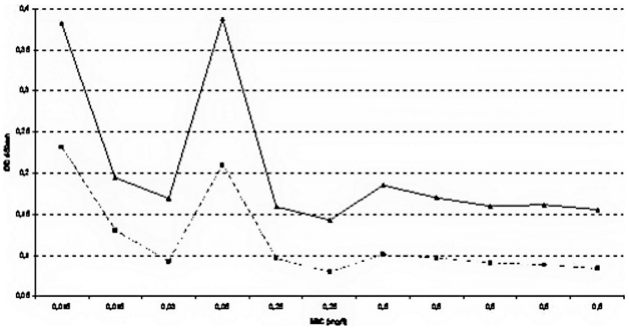
Objectives: To explore the in vitro effect of cefditoren (CDN) on slime production by slime-producing *Streptococcus pneumoniae* strains belonging to different serotypes and with different CDN MICs.
Methods: Eleven *S. pneumoniae* isolates with the following CDN MICs (mg/l) were used: 0.015 (serotypes 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-inoculated 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.



P1802 Macrophage interactions with biofilm-producing *Staphylococcus epidermidis*

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To compare immune responses of biofilm-producing *S. epidermidis* existing either in planktonic or in biofilm phase in terms of IL-12p40 production, bacterial adhesion on macrophages and resistance to phagocytosis process.
ATCC35983 and two clinical biofilm-positive, ica-positive *S. epidermidis* stains were used. Planktonic phase bacteria were obtained after 2 h incubation in Tryptic Soy Broth (TSB); whereas biofilm phase bacteria after 24 h 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 cultured on 96-well plates were incubated with biotinylated bacterial suspensions. For cytokine determination, macrophages cultured 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 ($7.6\pm0.02\times10^6$ for biofilm phase bacteria vs $3\pm0.06\times10^6$ CFU/macrophage 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) ($p < 0.05$).

Phase	% intracellular survival									
	Compared to initial inoculum				Compared to 2 h intracellular survival					
	20 min	60 min	90 min	120 min	4 h	12 h	24 h	48 h	3 days	5 days
Biofilm	39	31	27	15	66	40.2	24	1.5	0.8	0.05
Planktonic	3.64	2.3	1.3	0.4	11.4	2.05	1.8	0.4	0.11	0

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 mechanism of resistance of biofilm-associated infections to immune system responses.

P1803 The effect of different proteases on staphylococcal biofilm

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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 in 4 of 6 strains tested, is the more effective; in particular the effect seemed to be proportional to biofilm production. Moreover SPEP treatment showed a limited modification of surface protein patterns as demonstrated by SDS-PAGE and zymogram analysis, while treatment with serin-proteases induced a complete digestion of most of them. A correlation between the action of SPEP and agr-type of different bacterial strains is ongoing.
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* 6538P, 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

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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-bacteria was determined by quantification of gDNA copies of the housekeeping gene guanylate monokinase via quantitative PCR. The effect of delta-toxin was measured 24 hrs after administration unless otherwise specified.
Results: In vitro, 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

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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: Of 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*

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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

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, rnaIII, atl and icaA) in two isolates (COL, NRS149 – kindly supplied by NARSA) 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, rnaIII, atl and icaA mRNA.
Results: Our results show that the expression of sarA and rnaIII 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

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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.
Results: Six determinants (hla, hlb, fnbA, clfA, icaA, and agrII) were found to be predominant among these isolates. Enhanced biofilm formation was confirmed in hla-, hlb- 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-, hlb-, 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, hlb, and fnbA gene products.

P1808 Association of accessory gene regulator locus with biofilm formation and methicillin-resistance in *Staphylococcus aureus*

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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 with 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 MRSA 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 O_3 against biofilm

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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 from oxygen and preparation of ozonised water, has been applied in this study.
The aim of this study was to analyse bactericidal activity of ozonised water and gaseous O_3 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 37ºC for up to 72 h. Planctonic cells were removed and bacterial biofilm layer was treated with freshly obtained ozonised water or gaseous O_3. After various contact time of bacteria and ozone solution: from 30 sec to 4 min, and gaseous O_3: 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). Parallely, 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/glicyne 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 ug/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 O_3 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.

P1810 **Antimicrobial activity of crude eucalyptus oil against *Staphylococcus aureus*, MRSA, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* grown in planktonic and biofilm cultures**

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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/fungicidal concentrations of EO were determined 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 antiseptics in the prevention of HAI. Further studies are warranted.

P1811 **Activity of oritavancin against a clinical isolate of *Staphylococcus aureus* in in vitro biofilm models**

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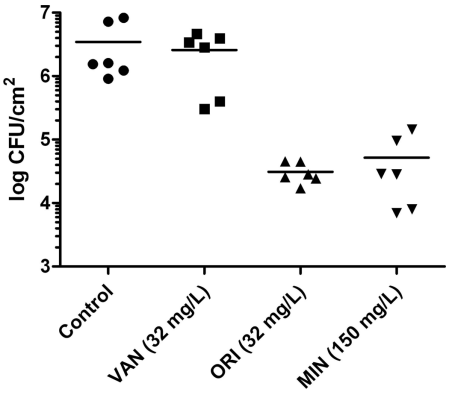
Objectives: Oritavancin (ORI) is a semi-synthetic lipoglycopeptide that is currently in clinical development for serious Gram-positive infections. The activity of ORI and comparator agents was determined against in vitro biofilms derived from a methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolate in two biofilm model systems.
Methods: A clinical isolate of MRSA isolated from an osteomyelitis infection was used to form 72-hour biofilms under low shear-forces in a drip-flow reactor (DFR) on hydroxyapatite-coated glass slides or in MBEC™ Physiology & Genetics Assay plates (Innovotech, Canada). Following exposure to ORI (32 mg/L), vancomycin (VAN; 32 mg/L) or minocycline (MIN; 150 mg/L) for 48 hours, DFR biofilm cell viability was determined by serial dilution plating. The minimal biofilm eradication concentration (MBEC) for the antibacterial agents was determined in the MBEC™ plates after 24 hours of exposure.
Results: DFR biofilm cell densities after 48-hour exposure to VAN, ORI and are shown in the figure (results from 3 independent experiments). ORI and the positive control MIN reduced biofilm cell density compared to control biofilms by 1.9 ± 0.5 and 1.9 ± 0.8 log, respectively ($P < 0.05$ vs. control for both drugs), whereas the effect of VAN was negligible. In the MBEC™ biofilm model, ORI sterilised the MRSA biofilms at MBEC = 8–32 mg/L whereas VAN and MIN did not and exhibited MBECs >128 mg/L.
Conclusion: These results demonstrate that ORI is active against in vitro biofilms of MRSA in different biofilm model systems and show promise for development of ORI as a therapy for biofilm-type infections.



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**

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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, 24 h old culture of *C. albicans* on Sabouraud agar was used to inoculate RPMI medium to give initial inoculum 1 to 5×10^6 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 2 h and free cells were removed by passing plain RPMI at the same rate for 2 h. 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 12 h (t1/2 of VOZ =6.5 h). 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.

P1813 **Interactions between triazoles and echinocandins against *Candida albicans* planktonic cells and biofilms**

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Objectives: *Candida albicans* (CA) is an important cause of endovascular infections that are related to foreign bodies. As antifungal triazoles are commonly administered concurrently with echinocandins, and as triazoles have been previously shown to have antifungal activity against *Candida* biofilms (BF) only at very high concentrations (>256 mg/L, Katragkou et al, AAC 2008; 52: 357), we studied the antifungal activity of voriconazole (VRC) against CA BF or planktonic cells (PL) in combination with two echinocandins, caspofungin (CAS) or anidulafungin (AND). Similarly, we studied the activity of posaconazole (PSC) in combination with the same two echinocandins.

Methods: CA-M61, a well-documented BF-producing CA strain, was grown in RPMI at 5×10^5 blastoconidia/mL on silicone disks in 96-well plates at 37ºC for 48 h in order to produce BF. PL were grown in YNB at 37ºC for 24 h. PL and BF were then incubated for 24 h with 2-fold dilutions of VRC or PSC (16–1024 mg/L), and CAS or AND (0–16 mg/L) alone or concurrently, VRC/PSC with CAS/AND in a checkerboard format. Fungal damage induced by antifungal agents to PL and BF was assessed by XTT assay. The interactions between VRC+AND, VRC+CAS, PSC+AND and PSC+CAS were analyzed using the Bliss model (Meletiadis et al, Med. Mycol. 2005; 43: 133). Synergy, antagonism or indifference was concluded when the observed fungal damage was significantly higher than, lower than or equal to the expected damage, respectively.
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.

P1814 Evaluation of triazole-echinocandin interactions against *Candida parapsilosis* planktonic cells and biofilms

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Objectives: *Candida parapsilosis* (CP) frequently causes nosocomial blood stream 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) only at very high concentrations (>256 mg/L, Katragkou et al, AAC 2008; 52: 357), little is known about the combined activity of these antifungal drug classes against CP BF. Therefore, we investigated the activities of two triazoles, voriconazole (VRC) and posaconazole (PSC), against CP BF or planktonic cells (PL) in combination with each of two echinocandins, caspofungin (CAS) and anidulafungin (AND).
Methods: CP-A71, a well documented BF-producing CP strain, was grown in RPMI at 5×10^5 blastoconidia/mL on silicone disks in 96-well plates at 37ºC for 48 h in order to produce BFs. PL of CP were grown in yeast nitrogen base growth media at 37ºC for 24 h. PL and BFs were then incubated for an additional 24 h with 2-fold dilutions of VRC or PSC (16–1024 mg/L) and CAS or AND (0–16 mg/L), alone or concurrently, PSC/VRC with AND/CAS in a checkerboard format. The degree of fungal damage induced on BF and PL was assessed by the XTT metabolic assay. The interactions between VRC+CAS, VRC+AND, PSC+CAS and PSC+AND were analyzed using the Bliss model (Meletiadis et al, Med. Mycol. 2005; 43: 133). Synergy, antagonism or indifference was concluded when the observed BF damage was significantly higher than, lower than or equal to the expected theoretical damage, respectively.
Results: The interaction between VRC at concentrations from 32 to 1024 mg/L and CAS at concentrations from 1 to 16 mg/L was antagonistic against CP PL but indifferent against CP BF. The other drug combinations (VRC+AND, PSC+CAS, PSC+AND) were indifferent against CP PL or BFs.
Conclusions: The antagonistic or indifferent interactions between triazoles and echinocandins, observed against *C. parapsilosis* PL or BFs at the concentrations studied, do not support the concurrent administration of these antifungal classes in cases of *C. parapsilosis* infections of vascular catheters.

P1815 Combined activities of antifungal azoles with calcineurin inhibitors against *Candida albicans* biofilms

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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 (TCR), 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 laser scanning microscopy (CLSM) and XTT assays were performed. For CLSM, BF were formed on silicone disks placed in 12-well plates at 37ºC 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 37ºC 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: 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).
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.

P1816 Bacterial adhesion on antibiotic-loaded polymethylmethacrylate bone cement

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Background: Polymethylmethacrylate (PMMA) bone cement, commonly used for the fixation of joint prostheses and also for orthopaedic devices, may be colonised by Gram positive cocci. The use of antibiotic-loaded PMMA should prevent bacterial colonisation of cement surfaces. Biofilm formation on bone cement could be more effectively reduced by incorporation of a second antibiotic The aim of our study was to evaluate the in vitro effects of gentamicin (G)- and vancomycin (V)-loaded PMMA cement specimens on the bacterial adhesion of multiresistant clinical isolates staphylococci.
Materials: Different strains of clinical isolates staphylococci (Met-R and Gent-R, and VRSA) and *E. coli* were studied. The PMMA specimens (discs), loaded with G (1.9%) or V (1.9%) or with their combination were placed singularly in Mueller-Hinton Broth inoculated with bacterial strains. After incubation at 37ºC for 24–72 hours, bacterial growth was determined by subcultures and optical density (OD540), and biofilm was stained with crystal violet. The biofilm PMMA-associated was measured spectrofotometrically. Antibiotic concentrations were determined by FPIA.

Results: Antibiotic-loaded specimens released high and inhibitory concentrations of G and V. G and V alone in PMMA cement reduced the bacterial adhesion of susceptible and intermediate-resistant Staphylococci. Only VRSA strain and *E. coli* were able to grow and to adhere to V-loaded specimens after 24 h. The combination exhibited synergistic bactericidal activity against all different multiresistant *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.

P1817 A scanning electron microscopy based quantitative method to evaluate plaque accumulation in patients undergoing different oral home care protocols

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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).

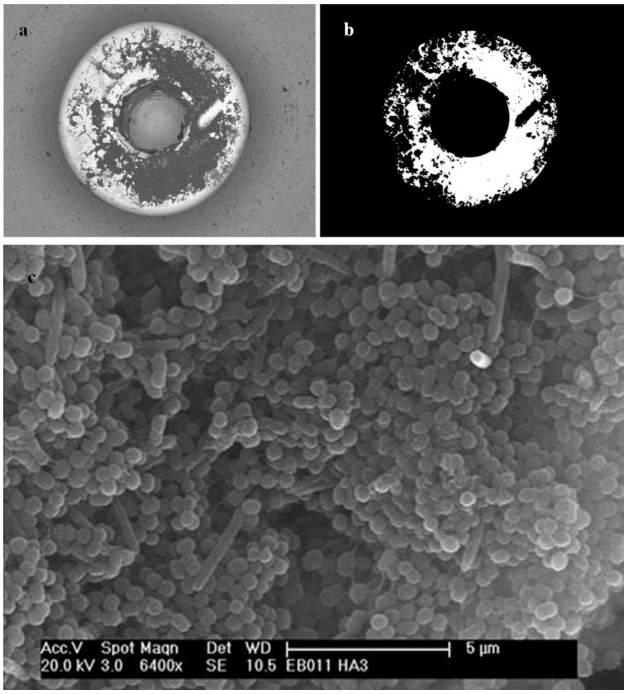

Figure 1. Plaque on healing abutment imaged by SEM in BSE mode: (a) raw and (b) thresholded image. (c) Bacterial morphologies in plaque biofilms at higher magnification.

Methods: The study was designed as a single blind randomised cross-over 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 from 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

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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).
Methods: *S. aureus* 6538P and *S. epidermidis* RP62A strains were used, reference strains for the biofilm formation. Determination of MIC and MBC for all natural compounds used was carried out. The influence on biofilm formation was assessed by a modified Christensen technique. The effect of the tested compounds on surface proteins and autolysins of the studied strains was evaluated by SDS-PAGE and zymogram, respectively. Surface proteins were extracted from bacteria cultured in the absence and in the presence of sub-inhibiting concentration of each compound.
Results: 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

particularly interesting for a possible application in biofilm infections in synergic therapy.

Molecular bacteriology – part 3

P1819 The role of CTX phage in the emergence of the *Vibrio cholerae* variants with different genomic organisations

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Objectives: Cholera toxin is encoded by the ctxAB operon in the genome of a filamentous bacteriophage (CTXtheta). 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 PstI 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 %17of 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.

P1820 The influence of enterocin B on the morphophysiology of *Escherichia coli*

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Objectives: Bacteriocins are the novel target for the search of new antimicrobibial agents different from antibiotics. Previously it was shown that enterococcal bacteriocin (enterocin B) is highly effective against Gram-positive bacteria (Moreno et al. 2006, Nilsen 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, 0002 mg/ml) were added on the surface of the solid medium (Tripticase 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 RFFI 06–04-48949.

P1821 Complementation in hypermutable *Escherichia coli* strains shows that mismatch repair and 8-oxoG pathway genes are not always responsible for mutator phenotype

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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 pGEMT-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 mutH or mutT genes could partially revert the MP, suggesting that a second order of selection by hitchhiking process can be possible.

P1822 Comparative distribution of phylogenetic groups, virulence genes and antimicrobial resistance in *Escherichia coli* isolated from blood, urine and faeces

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Objectives: We compared the distribution of phyolgenetic 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 (sfsS, fogG, 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, piperacillin/tazobactam, cefazolin, cefoxitin, cefotaxim, ceftazidime, cefepime, amikacin, gentamicin, tobramycin, tetracycline, ciprofloxacin, trimethoprim/sulfamethoxazole, imipenem were determined by VITEK 2 automated system (bioM?ieux, 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 sfaS 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 bacteraemia.

P1823 Enteric pathotypes of *Escherichia coli* involved in infectious diarrhoeal syndrome

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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 multitests media: TSI, MIU, MILF, Citrate and confirmated 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/VTEC (with one gene or association of 2–3 genes: eae, stxI, stxII, mdh), 0.2% typical STEC/EHEC (all 4 genes), 4.4% EAEC (agg gene), 0% EIEC and ETEC (genetic unconfirmed). There were no enteric pathogenic strains of *E. coli* isolated from HIV (+) patients.
Conclusions: 1. Pathotyps of *E. coli* was confirmed by genotypical methods, only in HIV(−) patients; in HIV(+) patients, ADS occured by other non-infectious pathophysiological 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

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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 asses 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 (bioMérieux). 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

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Objectives: Enteroaggregative *Escherichia coli* (EAEC) is an increasingly recognized enteric pathogen. It causes acute and persistent

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 asses the incidence, aetiology and course of patients hospitalised for gastro-enteritis (GEops 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 pic 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 GEops study.

P1826 Molecular characterisation of pUO-StVR2 variants belonging to a family of virulence-resistance hybrid plasmids of *Salmonella enterica* serovar Typhimurium originated from pSLT

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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-streptomycin/spectinomycin-sulphonamycin-tetracycline/blaOXA-1-catA1-aadA1-sul1-tet(B) pattern conferred by the plasmid to *S. Typhimurium*. The left and right flaking 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 Tn9- and Tn10-like transposons, respectively. In addition, pUO-StVR7 has gained a second integron (1000 bp/aadA22), apart from InH (2000 bp/blaOXA-1-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-dfrA10–3′CS element, which converts InH into a complex integron, and conferred additional resistance to trimethoprim. The insertion site of the foreign DNA, between the ccdAB 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

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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 assesed 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 significative 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

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Objectives: The microbiological diagnosis of periprosthetic joint infection (PJI) 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. PJI

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%).

Conclusions: 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.

P1829 Direct identification of aerobic Gram-positive cocci and Gram-negative rods from positive bioMerieux blood culture bottles by GenoType blood culture assay (HAIN)

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Objectives: Reliable and rapid identification of bacteria directly from blood cultures is important for the clinicians to help them decide on appropriate antibiotic therapy. The aim of this study was to evaluate a DNA strip assay (GenoType blood culture, Hain Lifescience) for the detection of the most important bacterial sepsis pathogens directly from positive blood cultures (bioMerieux BacT/Alert FA, SN and PF-bottles).

Methods: The material consisted of 100 positive blood cultures (92 patients, one quality control sample) out of which 152 positive bottles (FA and/or SN or PF and/or SN) were studied. Gram staining was carried out to decide whether to use GenoType BC GP (Gram positive cocci) or GN (Gram negative rods) test. The results were compared with the routine identification methods used in our laboratory. Aerobic bottles (FA and PF) contain charcoal which in blood culture vials may inhibit PCR reaction. To exclude the inhibitory effect of charcoal, blood samples were heated in 95ºC for 5 minutes before DNA extraction. All the samples regardless of the bottle type were heated and the test was performed with both heated and unheated samples.

Results: The GenoType BC test was easy to perform and required only a small amount of blood as starting material (15 µl). The results were available within 5 hours.

The heating of blood samples for 5 minutes in 95ºC before DNA extraction yielded more results compared to unheated samples. This was found, not only in FA and PF blood culture bottles which contain charcoal, but also in SN bottles which do not contain charcoal. In total we performed the Geno Type test from 152 heated and 152 unheated specimens. We yielded a result from 122/152 (80.3%) heated bottles and 107/152 (70.7%) from unheated bottles.

Four blood cultures (4/100) contained multiple pathogens. These were excluded from the data. Based on Gram staining 62/96 of the samples were Gram positive and 34/96 were Gram negative. Of the 96 positive blood cultures, seven (7.3%) were not identified by GenoType because the primers for these species are not included in the assay. The identification of the remaining 89 specimens (Table 1) was consistent with our reference method in 51/56 (91.1%) of Gram positive cocci and in 30/33 (90.9%) of Gram negative rods.

Conclusions: GenoType Blood culture kits provide fast and reliable results for the identification of the most important sepsis pathogens. Our results indicate that heating improves tests efficiency.

Table 1. Comparison between conventional methods and GenoType assay with either heated or unheated samples[a]

Isolate	Identification by conventional methods (n)	Consistent results by GenoType (n)	Contradictory results by GenoType (n)
Gram-positive cocci			
S. aureus	16	16	
S. epidermidis	9	9	
S. hominis	6	6	
S. warneri	2	1	1, No result by GenoType
Staphylococcus spp.	1	0	1, No result by GenoType
Str. pneumoniae	7	7	
Str. pyogenes A	4	4	
Str. G	2	2	
Str. anginosus group	3	2	1, Strain identified as *Str. mitis/oralis* by GenoType
Str. mitis	1	0	1, No result by GenoType
E. faecium	2	1	1, No result by GenoType
E. faecalis	3	3	
Total	56	51	5
Gram-negative rods			
E. coli	21	21	
K. pneumoniae	5	4	1, Strain identified as *K. oxytoca* by GenoType
P. mirabilis	1	1	
Ps. aeruginosa	4	3	1, No result by GenoTtype
Enterobacter aerogenes	1	1	
Salmonella Newport	1	0	1, Strain identified as *S. enteritidis/typhimurium* by GenoType
Total	33	30	3

[a] 71/89 (79.8%) of the isolates were identified from heated and unheated bottles.
10/89 (11.2%) of the isolates were identified only from heated bottles.
3/89 (3.4%) of the isolates were identified only from unheated bottles.
5/89 (5.6%) of the isolates were not identified either from heated or unheated bottles.

P1830 Usefulness of PCR on normally sterile body fluids for the detection of fastidious bacteria

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Objectives: Molecular techniques to detect difficult to grow microorganisms in the clinical microbiology laboratory have become routine. Their contribution to patient care needs to be addressed. Thus, we assessed here the performance of PCR done on various types of normally sterile body fluids (i.e articular, pericardic, peritoneal and pleural fluids) for the detection of fastidious bacteria. These bacteria frequently remain undetected as they grow poorly or not at all on conventional culture media.

Methods: 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.

Results: On the 1247 samples, 1370 PCR tests were performed. Eighty-three samples were positive for one of the PCR corresponding to 7% of the samples. Eleven samples (0.9%) contained inhibitors. In 4 to 14% of the various types of samples a positive PCR result could document the infection, with *M. tuberculosis* complex PCR (4%), bacterial broad-range PCR (14%), and *C. trachomatis* (12%), being the most productive tests (see Table).

For the other PCR results, 2/39 articular fluids were positive for *B. burgdorferi*, and 2/37 for *N. gonorrhoeae*; 1/38 peritoneal fluid was positive for *N. gonorrhoeae*; 1/13 pleural fluid was positive for *L. pneumophila* and 1/20 for *M. pneumoniae*. No specific samples were positive for *C. pneumoniae* nor for Mycobacteria spp other then *M. tuberculosis* complex.

Conclusion: Carefully indicated molecular methods could bring a positive 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.

Fluids	Samples[a]								
	All (n = 1247)	All + (n = 83)	% of +	+ for *M. tuberculosis*/ total no. of samples tested	% of +	+ for bacterial broad-range PCR/ total no. of samples tested	% of +	+ for *C. trachomatis*/ total no. of samples tested	% of +
Articular	249	21	8%	5/91	5%	12/97	12%	0/20	0%
Pericardic	73	6	8%	5/67	7%	1/11	9%	0/0	–
Peritoneal	161	22	14%	0/21	0%	2/6	33%	19/134	14%
Pleural	764	34	4%	23/699	3%	9/62	15%	0/0	–
Total	1247	83	7%	33/878	4%	24/176	14%	19/154	12%

[a] +, positive.

P1831 Performance of PCR on adenopathies for the detection of fastidious pathogens

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Objectives: Molecular tools have proven to be extremely useful in the clinical diagnostic laboratory, especially for detecting difficult to grow microorganisms. Although technical issues such as reproducibility, sensitivity, and specificity of these tests are important, costs and potential contribution to patient care are also of concern and need to be addressed. Here, we assessed the performance of PCR on adenopathies, for the detection of fastidious bacteria, which frequently remain undetected as they grow poorly or not at all on conventional culture media.
Methods: A total of 377 samples (322 fresh biopsies and 55 paraffin-embedded adenopathies) were investigated by 3 different PCR in our molecular diagnostic laboratory between 2000 and 2008. These PCR included 2 in house real-time TaqMan PCR specific for *Bartonella henselae* and *Mycobacterium tuberculosis*.) and a bacterial broad-range PCR with classic amplification of the 16S rDNA followed by sequencing of the amplified product.
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).

Adenopathies	Samples						
	All (n = 377)	All positive (n = 92)	% of +	Positive for *B. henselae*/total no. of samples tested	% of +	Positive for *M. tuberculosis*/ total no. of samples tested	% of +
Freshly biopsied	322	73	23%	11/108	10%	60/248	24%
Paraffin-embedded	55	19	35%	1/14	7%	18/53	34%
Total	377	92	24%	12/122	10%	78/301	26%

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?

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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 6 h. 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 50 y, range 34–80 y, 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

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Background: Blood cultures (BC) identify the aetiology of sepsis in only a minority of patients and results are usually available $\geqslant$1 days later. More rapid and sensitive tools 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 6 h.
Methods: In this prospective ongoing study, we included unselected adult patients presenting to the emergency room with suspected sepsis, defined as core temperature >38.3ºC or <36.0ºC and $\geqslant$1 additional SIRS criterion. BC (BacT/ALERT, FA/FN, bioMérieux) and SF were simultaneously drawn at presentation and 0.5–2 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 pulmonal (34%), urinary tract (23%), abdominal (12%), skin (9%), ENT (7%), musculoskeletal (3%), 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 6–45 h). 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.
Conclusion: In patients with confirmed sepsis, BC detected the causative organism in 21 (28%) and SF in 17 (23%). BC missed the causing 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.

Parameter	Blood culture (BC)	SeptiFast® (SF)
Sensitivity	28%	23%
Specificity	86%	100%
Positive predictive value	91%	100%
Negative predictive value	19%	20%
Accuracy	38%	35%

P1834 Contribution of the real-time polymerase chain reaction from heart valve tissue for the diagnosis of infective endocarditis

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Objectives: 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).
Method: We analyzed 82 valves obtained from patients with definite IE using the modified Duke criteria. For each valve, we have performed conventional cultivation method and real-time PCR assay using the Smart Cycler® II (Cepheid, Instrumentation Laboratory) with the researches of *Bartonella, Tropheryma whipplei, Coxiella burnettii, Chlamydia pneumoniae* and *Mycoplasma pneumoniae* and universal gene encoding 16SrRNA (with primers 91E and 13BS). Amplification products obtained with the universal primers were sequenced and analyzed with the BIBI GenBank.
Results: On the 82 valves, we isolated bacterial strains from 22 (26.8%) by conventional culture and we obtained an amplification product with the universal RT-PCR for 18 (22%) valves with a concordance in 5 cases. The RT-PCR allowed to detect a bacterial origin of the IE in 13 new cases. When culture and universal RT-PCR were associated, a bacterial identification was obtained in 35 cases, corresponding to 42.7% of patients (see Table).

Results of conventional culture and universal RT-PCR

	Positive PCRs	Negative PCRs	Total
Positive cultures	5	17	22
Negative cultures	13	47	60
Total	18	64	82

In two supplementary cases, *T. whipplei* was identified by specific in-house RT-PCR, whereas all other specific RT-PCRs were negative.
Conclusion: RT-PCRs appear as complementary methods of the conventional culture for the diagnosis of IE from explanted heart valve tissues notably for patients without blood cultures or with negative blood cultures. It must be noted that the culture remains the reference method since it allow to obtain the antimicrobial susceptibility of strains from positive cultures.

P1835 The Mobidiag Prove-it sepsis PCR and microarray platform: a 2-centre study designed to validate a system for speciating blood culture bacterial isolates within 3 hours

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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.
Conclusions: 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.

P1836 Comparison of molecular biology-based methods and culture methods in diagnosing infectious endophthalmitis

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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 eubacterial 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.

P1837 Molecular charecterisation of *S. pneumoniae* with reduced susceptibility to macrolides

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Despite of the gained results in treatment and diagnostics, *S. pneumoniae* is still remaining the actual pathogen of pneumonias, meningitids etc. Macrolide resistance has been reported to be high among pneumococci in Asian countries, but there is no information on macrolide resistance of *Streptococcus pneumoniae* at the Far East of Russia. The main objective of this study was to characterise isolates of *S. pneumoniae* with reduced susceptibility to macrolides by different typing methods and to evaluate possible ways of clonal dissemination.
Methods: Isolated 48 macrolide non-susceptible strains of pneumococcus were serotyped, PCR with macrolide resistance primers, molecular typed by pulsed-field gel electrophoresis (PFGE) and automated ribotyped. Antimicrobial agents resistance was studied by NCCLS standards.
Results: A total of 35.82% (48 of 134 strains) of the *S. pneumoniae* strains were resistant to erythromycin with a MIC of 1.0 g/ml. Of these, 31.25% (15of 48) showed an MLSB phenotype with erythromycin and clindamycin; 66.6% showed resistance to erythromycin alone (M phenotype). One isolate was postive with both ermB and mefE primers. Of the isolates expressing the MLSB phenotype, only the ermB gene was detected in 86.63% (13 strains of 15) of the isolates by PCR. Two isolates were repeatedly negative on testing for ermB but were positive for ermA gene. Before 2000, it was recorded that among the erythromycin-resistant *S. pneumoniae* isolates, the majority (78%) had an ML phenotype and 22% had an M phenotype. The isolates were distributed among four major serotypes: 23F (29.1%), 14 (35.4%), 18C (25%), 6B (12.5%). However, distinct molecular patterns were detected among isolates with a unique serotype. Six and eight PFGE patterns were identified among isolates with serotype 23F and 18C, respectively. When PFGE and automated ribotyping analyses were combined, five clusters were identified. The largest cluster (14 isolates) was represented by isolates heterogeneous but six were highly similar to the Spanish multidrug-resistant 23F clone although possibly related to the Taiwan multidrug-resistant 23F clone.
Conclusion: New techniques allowed us to evaluate the clonal dissemination of *S. pneumoniae* strains and they should be more wide included to the epidemiology surveillance schemes.

P1838 Invasive and pharyngeal emm-3 *Streptococcus pyogenes* infections in Norway: a molecular genetic analysis

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Group A streptococci (GAS) are important pathogens worldwide, mostly causing mild forms of disease, such as impetigo and pharyngitis, but invasive and life-threatening forms of disease, such as necrotising fasciitis (NF) and streptococcal toxic shock syndrome may occur. After the introduction of penicillin GAS disease almost disappeared, but since the late 1980 s it has re-emerged, showing a marked increase both in incidence and severity in Norway. Moreover, strains with the emm-3 variant of the gene coding for the M-protein, a major virulence factor, are often associated with higher mortality rates and higher frequencies of NF than are other strains.
Objectives: To better understand the role of emm-3 GAS in disease production 128 emm-3 pharyngeal and invasive isolates from 1988–2003 were analyzed with respect to their content of prophages which harbours superantigens and other important virulence factors, and for the *Streptococcus* collagen-like protein B (SclB) which is associated with NF.
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-3.1 and prophage profile, phi-G3.01. In 1993 sclB 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 SclB was highly modular mainly consisting of two novel motifs; M3CSMR-1 and -2, organised into unique motif-patterns for most of the strains. A third motif M3CSMR-3 was also found for eleven strains, nine 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 sclB 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 sclB was found to be consisting of two novel emm-3 specific conserved motif repeats, displaying highly variable motif patterns.

P1839 Restoration of the wild-type phenotypes in *Streptococcus pyogenes* rgg mutant strains depends on the expression level of Rgg transcriptional regulator

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Objectives: The clinical outcomes of *Streptococcus pyogenes* infections range from mild pharyngitis to severe toxic shock syndrome and necrotising fasciitis. The expression of *S. pyogenes* virulence factors is coordinately regulated transcriptionally, translationally and post-translationally. Rgg is a global transcriptional regulatory protein necessary for SpeB expression. In a strain-specific manner, Rgg additionally affects numerous phenotypes such as virulence, tolerance to thermal and oxidative stress, fermentation of amino acids during the exponential phase of growth, and the ability to grow in enriched and chemically defined media, among others. Given the potential differences in expression level of Rgg in *S. pyogenes* strains, it was of interest to determine if a correlation existed between the Rgg-regulated phenotypes and the level of Rgg expression.
Methods: The wild-type *S. pyogenes* strains NZ131, SF370, and their rgg isogenic mutants were used. Rgg expressing plasmids were constructed by methods of genetic engineering. Complementation of the rgg mutants was done episomally and chromosomally under inducible promoter and chromosomally under native rgg promoter. Chromosomal complementation was done by the insertional mutagenesis. Phenotypes of all the strains were analyzed by the methods of biochemistry and microbiology. Gene expression levels were assessed by quantitative RT-PCR.
Results: The rgg gene was cloned into pMSP3535 vector under control of a nisin-inducible promoter and introduced into the rgg mutant strains resulting in episomal complementation of the rgg gene. Alternatively, the rgg gene was cloned into a suicide vector, which was unable to replicate in streptococci. Subsequently, an intact rgg gene was chromosomally restored either under inducible promoter or native rgg promoter. Restoration of rgg in the chromosome under the control of native promoter restored wild-type phenotypes, as determined by qRT-PCR and biochemical assays. In contrast, both episomal complementation and chromosomal complementation of rgg under control of an inducible promoter, resulting in over-expression of rgg, did not restore any of metabolic properties associated with the wild-type strain.
Conclusion: Rgg transcriptional activity depends on the rgg expression level. Given the importance of the rgg level in controlling virulence factor expression in *S. pyogenes*, further characterisation of the mechanism of Rgg-mediated gene transcription is necessary.

P1840 Inactivation of the transcriptional regulator mutR gene affects virulent phenotype of *Streptococcus pyogenes*

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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 1×10^8 no 5×10^8 CFU/animal resulted in the death of laboratory mice.
Conclusion: The MutR transcriptional regulator plays an important role in the virulence of *S. pyogenes*.

P1841 Comparison of Gene Xpert real-time PCR and various culture methods for the detection of group B *Streptococcus* in rectovaginal samples from ante-natal women

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Group B Streptococci (GBS) is an important cause of maternal and neonatal infection. The Gene Xpert 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 Gene Xpert. Low vaginal/rectal GBS screening of 102 antenatal women, using a double-swab, were compared using the Gene Xpert 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 onto the various agars 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 absence of GBS in ante-natal women and may be useful for rapid intrapartum testing.

P1842 Evaluation of two real-time PCR methods to direct detection of group B streptococci against conventional chromogenic cultures

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Objectives: Group B streptococcal disease remains a leading infectious cause of morbidity and mortality among newborns. Asymptomatic carriage of group B streptococci (GBS) in the maternal genitourinary tract or gastrointestinal tract commonly leads to colonisation of the neonate. Real-time PCR (PCR) methods that offer the ability to detect GBS colonisation within 1 h of sample receipt have also been developed. The aim of this study was to assess the use of these 2 new molecular methods in comparison with conventional culture methods for the isolation of GBS from vaginal-rectal swabs.
Methods: A total of 103 consecutive vaginal-rectal swabs from distinct pregnant women were included in this study. Any red colonies on Granada Agar agar were regarded as presumptive GBS. Such colonies were confirmed as GBS using latex agglutination for group B antigen. Two commercial PCR was tested: i) BD GeneOhm StrepB Assay (Becton Dickinson, USA) based in the amplification of cfb gene sequence of GBS and fluorogenic target-specific hybridisation for the detection of the amplified DNA; and ii) Speed-Oligo Group B *Streptococcus* (Vircell, Spain), that use a real-time PCR method and detection of DNA through hybridisation in a dipstick device based in reactive lines with colloidal gold technology. All methods follow the manufacturing recommendations.
Results: Twenty-one GBS strains could be isolated by culture from 103 samples. BD GeneOhm StrepB Assay detects DNA in 16 samples with positive culture and one sample with negative culture. However, Speed-Oligo Group B *Streptococcus* could detect DNA in 19 samples with culture positive and 4 samples with negative culture. Sensitivity, specificity, positive and negative predictive values was respectively 76.1, 98.7, 94.1, and 94.1 for GeneOhm versus 90.4, 95.1, 82.6, and 97.5 for Speed-Oligo. Analysing the discrepancies there was a close correlation between CFU in the culture and false negative in molecular test. In false-positive we cannot exclude the further possibility that PCR was detecting the presence of nonviable.
Conclusions: The PCR assay is rapid (<1 h) and is, therefore, particularly useful if testing needs to be performed during labour. This is an attractive option because colonisation with GBS may be transient, and results achieved weeks before labour may no longer be relevant at time of delivery. Although routine use of PCR during labour would require a local availability of a 24-h service, and a greater expense per test.

P1843 **Inactivation of DNA-binding response regulator Sak189 abrogates β-antigen expression and affects virulence of *Streptococcus agalactiae***

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Objectives: *Streptococcus agalactiae* (group B streptococcus) is able to colonise numerous tissues and to cause variety of diseases in human and animals. This bacterium rapidly adapts to changing environmental conditions employing different mechanisms of gene regulation. Recently, putative two-component regulatory system genes encoding for sensor histidine kinase (sak188) and DNA-binding response regulator (sak189) were identified in *S. agalactiae*. They were located on pathogenicity island downstream the bac gene encoding for β-antigen with IgA binding capacity, which was supposed to be important virulence factor. Given their adjacent location in the genome, we suggested that Sak188/Sak189 system influenced β-antigen expression and virulence properties in *S. agalactiae*.
Methods: *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 analyzed 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 inactivate sak188 and sak189 genes 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.

P1844 **Characterisation of group G streptococcal strains recovered from humans**

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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, spegg, smeZ). 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 spegg and smeZ using primers that anneal to structural gene sequences. All strains were susceptible to penicillin (MIC $\leqslant$ 0.063 mg/l), ampicillin (MIC $\leqslant$ 0.125 mg/l), ampicillin/sulbactam (MIC $\leqslant$ 0.125 mg/l), cefatotin (MIC $\leqslant$ 0.125 mg/l), nitrofurantoin (MIC $\leqslant$ 16 mg/l), teicoplanin (MIC $\leqslant$ 0.5 mg/l) and vancomycin (MIC $\leqslant$ 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.

P1845 **Emm-types of group A and G streptococci: invasive and noninvasive strains**

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Objectives: This study aims to characterise molecular properties of invasive and noninvasive group A (GAS) and group G streptococci (GGS). We compare strains isolated from superficial and invasive infections to see if tissue specificity is found.
Methods: During a six month period, 122 GAS and 138 GGS strains were collected in Pirkanmaa Health District from patients with various streptococcal infections. Each month approximately ten first clinical throat and skin isolates were selected amongst GAS and GGS isolates for analysis. In addition, all blood and deep tissue isolates were collected. Altogether, we collected 124 throat (62 GAS, 62 GGS), 101 skin (45 GAS, 56 GGS), 11 abscess (6 GAS, 5 GGS) and 22 blood isolates (8 GAS, 14 GGS), one bursa isolate (GAS) and one deep tissue isolate (GGS). All strains were characterised using emm typing.
Results: Among GAS 19 different emm types were found. Emm1 and emm28 were the most prevalent types (23%, 21%, respectively). Other common types among GAS were emm12 (12%), emm77 (12%) emm89.0 (12%). Emm77 was found more often on skin than other sites ($p = 0.005$) whereas emm1 and emm12 were more often found on throat than other sites ($p = 0.017$ and $p = 0.025$, respectively). Among GGS we found 18 different emm-types. Typing of seven GGS strains is still ongoing. The most prevalent emm types among GGS were stG480 (25%), stG643 (20%) and stC6979 (10%).
Conclusions: Several emm types were found from GAS and GGS. Most of the emm types are found from strains isolated from different infection sites. In GGS no association between emm types and isolation site was detected. In GAS emm77 associated with skin infections and emm1 and emm12 with throat infections. Emm typing may not be the best method to determine the tissue specificity of the streptococci strains.

Lipoglycopeptides

P1846 **Telavancin activity against *S. aureus* and coagulase-negative staphylococci collected from clinical infections in Europe (2007–2008)**

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Objectives: To assess the activity of telavancin, a potent investigational lipoglycopeptide agent with a multifunctional mechanism of action, and rapid bactericidal 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.

Organism (no. tested)	No. of strains (cumulative %) inhibited at telavancin MIC (mg/L) of:						
	⩽0.015	0.03	0.06	0.12	0.25	0.5	1
SA (5726)	–	16 (0.3)	382 (7.0)	3722 (72.0)	1574 (99.4)	32 (100.0)	–
OX-S (4150)	–	14 (0.3)	251 (6.4)	2765 (73.0)	1099 (99.5)	21 (100.0)	–
OX-R (1576)	–	2 (0.1)	131 (8.4)	957 (69.2)	475 (99.3)	11 (100.0)	–
CoNS (1808)	12 (0.7)	13 (1.4)	176 (11.1)	1003 (66.6)	566 (97.9)	38 (100.0)	–
OX-S (420)	2 (0.5)	9 (2.6)	56 (16.0)	236 (72.1)	113 (99.1)	4 (100.0)	–
OX-R (1388)	10 (0.7)	4 (1.0)	120 (9.7)	767 (64.9)	453 (97.6)	34 (100.0)	–

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.

P1847 Antimicrobial activity of telavancin tested against streptococcal species isolates collected from European medical centres (2007–2008)

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Objectives: To determine the antimicrobial potency and spectrum of activity of telavancin and key comparator agents against β-haemolytic streptococci (BST), *S. pneumoniae* (SPN) and viridans group streptococci (VGS) recovered from patient infections in European medical centres. Telavancin is a potent investigational lipoglycopeptide agent possessing a multifunctional mechanism of action and displaying rapid bactericidal activity against aerobic and anaerobic Gram-positive bacterial pathogens.
Methods: A total of 2,856 streptococcal clinical isolates including BST (820), SPN (1714) and VGS (285) were collected in 2007–2008 from 28 medical centres in 11 European countries, Turkey (2 sites) and Israel (1 site) as part of a Telavancin Surveillance Program. Susceptibility (S) testing for all strains was performed using the CLSI broth microdilution method (M7-A7; 2006) at a central processing laboratory.
Results: Against BST, telavancin was 16-fold more potent (MIC50; 0.03 vs. 0.5 mg/L) than vancomycin (VAN). Telavancin was more active against Group A BST (MIC90, 0.03 mg/L) compared to Groups G and B BST (0.06 and 0.12 mg/L, respectively). All VGS and SPN strains were inhibited by telavancin at ⩽0.12 mg/L. Telavancin (MIC90, 0.06 mg/L) was also 16-fold more active than VAN (MIC90, 1 mg/L) against VGS strains. Telavancin results for the 4 major species of VGS were analyzed separately and no MIC variation was observed among these organisms. SPN strains from Italy and Spain had telavancin MIC results (MIC50/90; 0.03/0.06 mg/L) slightly higher compared to France, Germany and Ireland/United Kingdom (MIC50/90; ⩽0.015/0.03 mg/L). Similarly, SPN isolates from community-acquired respiratory tract infections exhibited telavancin MIC values slightly higher (MIC50/90, 0.06/0.12 mg/L) than those from bloodstream infections (MIC50/90, 0.03/0.03 mg/L). Overall, 15.3 and 1.4% of SPN were resistant to penicillin and levofloxacin, respectively. Telavancin activity is summarised in the Table.
Conclusions: Telavancin was highly active against BST, VGS and SPN streptococcal strains isolated from European medical centres cultured in the past two years (2007–2008); all streptococci were inhibited at ⩽0.25 mg/L. Telavancin was four- to 16-fold more potent than vancomycin overall, based on both MIC50 and MIC90 values. Continued monitoring for resistance emergence among Gram-positive streptococcal species is warranted to detect possible threats to this potent new therapeutic agent.

Organism (no. tested)	MIC (mg/L)		Cumulative % inhibited at MIC (mg/L):				
	50%	90%	⩽0.015	0.03	0.06	0.12	0.25
BST (820)	0.03	0.12	13.5	57.2	87.9	99.4	100.0
Group A (341)	0.03	0.03	27.9	93.0	99.7	100.0	–
Group B (330)	0.06	0.12	0.0	11.8	72.4	98.5	100.0
SPN (1714)	0.03	0.03	49.5	95.3	99.8	100.0	–
VGS (285)	0.03	0.06	14.2	69.8	95.1	100.0	–

P1848 Telavancin antimicrobial activity when tested against enterococci and uncommonly isolated Gram-positive species (European sample for 2007–2008)

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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:

Organism (no. tested)	MIC (mg/L)		Cum. % inhibited at MIC (mg/L):					
	50%	90%	⩽0.06	0.12	0.25	0.5	1	2
VSEF (1,320)	0.25	0.5	1.1	16.6	72.2	99.6	100.0	–
VREF (17)	>2	>2	–	–	5.9	17.7	17.7	23.6
VSEFM (543)	⩽0.06	0.12	71.3	98.1	100.0	–	–	–
VREFM (216)	2	>2	17.1	23.1	26.3	27.7	35.1	81.5
E. avium (24)	⩽0.06	0.12	70.8	91.6	91.6	91.6	100.0	–
E. gallinarum (16)	0.5	1	12.5	43.8	43.8	56.5	100.0	–

Telavancin was more active against EFM (four-fold) then EF. Vancomycin (VAN) resistance (R) adversely affected telavancin MIC results for both EF and EFM increasing MIC90 results to >2 mg/L, however, VAN-susceptible (S) EF (MIC90, 0.5 mg/L) and EFM (MIC90, 0.25 mg/L) were telavancin-S. The telavancin MIC90 (mg/L) for *E. avium*, *E. gallinarum*, BC, LM, ML and CS were 0.12, 1, 0.12, ⩽0.06, ⩽0.06 and ⩽0.06 mg/L, respectively. Against all strains, site of infection and geography had no influence on telavancin potency (MIC50, 0.25 mg/L). VAN-R rates varied from 0.0–0.8% (Spain, France) to 22.9% (UK) with VAN-A patterns ranging from 0.0 (France) to 98.7% (UK). High-level R for gentamicin (35.9%) and streptomycin (43.2% was also noted).
Conclusions: Among EU isolates, telavancin was more potent and provided wider coverage of EFM than EF strains. Continued monitoring of this new lipoglycopeptide would be prudent to detect any emerging R and to establish the role of this novel, potent agent against *Enterococcus* spp. infections in EU.

P1849 Efficacy of oritavancin at single or infrequent doses for the treatment of complicated skin and skin-structure infections

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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 doses of Ori as potentially efficacious for cSSSI.
Methods: Phase 2, multi-centre, double blind, randomised, parallel, active comparator study of patients with Gram-positive cSSSI. Patients were randomised to comparator Ori daily-dose, Ori single dose (1200 mg on Day 1), or Ori infrequent dose (800 mg on Day 1/optional 400 mg on Day 5). IV placebo was given to maintain blind. Signs and symptoms of cSSSI, blood/cSSSI cultures, and safety measures were assessed at predetermined times. Clinical efficacy was assessed at end of therapy, test of cure (TOC), & late follow-up (LFU).
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 dosing groups and all were well tolerated.

Pop (N)	TOC Cure Rate (%)		
	MD Ori 200 mg daily for 3–7 days	SD Ori 1200 mg D1 (90% CI)*	ID Ori 800 mg D1/400 mg D5 (90% CI)*
ITT (300)	72.4	81.8 (−1.7, 17.8)	78.2 (−5.8, 14.6)
CE (228)	72.4	81.5 (−2.5, 18.2)	77.5 (−6.8, 15.4)
MRSA CE (82)	73.9	74.3 (−18.8, 18.7)	83.3 (−5.7, 28.0)
ME (161)	69.1	79.3 (−5.2, 20.0)	81.3 (−2.9, 22.6)
LFU Relapse (%)			
CE (160)	0	1.6	3.7

*CI is based on estimated difference in response rate between patients in MD vs. SD or ID using Mantel–Haenszel method stratified by disease.

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.

P1850 Oritavancin in the treatment of immunocompromised patients with complicated skin and skin-structure infections

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Objectives: Oritavancin, a novel semisynthetic lipoglycopeptide with activity against a wide range of Gram-positive bacteria, including vancomycin-resistant staphylococci and enterococci, is presently under regulatory review for the treatment of complicated skin and skin structure infections (cSSSI). Immunocompromised patients are a vulnerable population who run the risk of increased morbidity and lower cure rates compared to immunocompetent patients.
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 cSSSI. Test-of-cure (TOC) occurred between Days 21 to 35. Described here are results from the group of patients considered to be immunocompromised at study entry. The immunocompromised group includes patients with baseline HIV/AIDS, AIDS, neutropenia, adverse events of neutropenia or those taking immunosuppressive concomitant medications.
Results: 1763 patients received study medication, of which 85 were immunocompromised. Clinical cure rates in non-immunocompromised patients in the clinically evaluable (CE) population at TOC were 76.9% (688/895) in ORI compared to 76.1% (337/443) for VAN. Similar response rates were observed in the immunocompromised group of patients and clinical cure rates in the CE population at TOC were 77.3% (34/44) in ORI compared to 66.7% (10/15) for VAN. Overall, more immunocompromised patients had at least one AE (78.0% for ORI and 76.9% for VAN) than patients who were immunocompetent (52.2% for ORI and 61.7% for VAN). Laboratory changes were similar between ORI and VAN treatment groups in patients with and without immunocompromise.
Conclusions: Oritavancin is a novel lipoglycopeptide with demonstrated efficacy and a favourable safety profile with short course therapy (3–7 days) in immunocompromised patients in two phase 3 studies of cSSSI.

P1851 Oritavancin in the treatment of diabetic patients with complicated skin and skin-structure infections

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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 existent 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 cephalexin. 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 12 h, 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% (125/201) 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.
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 in patients with diabetes.

P1852 Hepatic insufficiency and outcomes in patients with complicated skin and skin-structure infections treated with oritavancin

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Objective: Oritavancin (ORI) is a novel semisynthetic lipoglycopeptide with activity against a wide range of Gram-positive bacteria, including those resistant to currently existent 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 hepatically 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.

P1853 Safety of oritavancin versus vancomycin for treatment of patients with complicated skin and skin-structure infections

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Objective: Oritavancin (ORI) is a novel semisynthetic lipoglycopeptide with activity against a wide range of Gram-positive bacteria. Two randomised, double-blind, phase 3 studies compared the safety of ORI/placebo to VAN/cephalexin in patients treated for complicated skin and skin structure infections (cSSSI).
Methods: Patients randomised to ORI received 3–7 days of IV drug then 7–11days of IV/oral placebo. Patients randomised to vancomycin (VAN) received 3–14days of IV drug then 0–11days of oral cephalexin. 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 12 h, dose-adjusted based on creatinine clearance. We integrated safety data for analyses of adverse event incidence, clinical laboratory and vital signs changes.
Results: 1763 patients received study drug; 1173 ORI and 590 VAN. Significantly lower percentages of ORI versus VAN patients had $\geqslant$1 treatment-emergent adverse event and adverse events leading to study drug discontinuation. Lower but not significantly different percentages of ORI versus VAN patients died or had serious adverse events. Also, significantly ($p < 0.05$) lower percentages of ORI versus VAN patients had treatment emergent adverse events potentially related to histamine-like infusion reactions (pruritus, erythema, pruritus generalised, flushing, red man syndrome, urticaria, infusion site pruritus). Comparable percentages of ORI versus VAN patients had other treatment emergent adverse events potentially related to glycopeptides (infusion site pain, infusion site phlebitis, phlebitis, infusion site thrombosis, infusion site erythema). Neither treatment showed clinically relevant laboratory or vital signs changes.

Event type	Number* (%) with event		p-value
	ORI (N = 1173)	VAN (N = 590)	ORI vs VAN
TEAE	628 (53.5)	368 (62.4)	<0.001
SAE	107 (9.1)	67 (11.4)	0.150
AE Leading to SD DC	35 (3.0)	34 (5.8)	0.006
Death	19 (1.6)	12 (2.0)	0.566

*Except death includes pts with $\geqslant$1 event.

Conclusions: ORI as short-course therapy (3–7days) is safe and well tolerated in patients with cSSSI and has a favourable benefit-risk profile versus VAN.

P1854 Evaluation of oritavancin activity in vitro in the presence of human and mouse serum

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Objectives: Oritavancin (ORI), a semisynthetic lipoglycopeptide, exerts bactericidal activity against Gram-positive bacteria including drug-resistant strains. ORI is highly bound to human plasma components, principally serum albumin. It is generally held that the free fraction of an antibiotic in plasma is predictive of its activity. We therefore sought to compare the binding of ORI to human (hu) serum (SER) relative to mouse (mo) SER in vitro to support pharmacodynamics studies.
Methods: Avid binding of ORI to filtration and dialysis membranes renders most protein binding methodologies unsuitable. We therefore quantified MIC shifts (from arithmetic drug dilutions) and area under the inhibitory curve (AUIC) shifts (from time-kill assays) in the presence of SER compared to SER ultrafiltrate (ULTRA; free of albumin) to estimate ORI binding to serum. Pooled huSER and moSER were from Equitech-Bio (Kerrville, TX). ULTRA from both species was prepared using Amicon filtration units (50k MW cutoff). ORI stock solutions were prepared following CLSI M100-S18. Staphylococcus aureus ATCC 29213 was used as the test isolate at a final inoculum of ~5×105 CFU/mL in both MIC and AUIC studies.
Results: Mean ORI MICs in huSER and huULTRA were 0.78 ± 0.32 mg/mL and 0.13 ± 0.04 mg/L, respectively. Mean ORI MICs in moSER and moULTRA were 0.54 ± 0.05 and 0.08 ± 0.004 mg/L, respectively. The resulting estimated mean serum protein binding value for ORI to huSER (81.6%) is significantly different from that observed for moSER (85.3%) (Mann-Whitney U test; two-sided $P = 0.017$). From time kill-based AUIC studies the mean binding of ORI to huSER (64.3 ± 2.9%) and to moSER (67.8 ± 1.3%) is not significantly different (unpaired t-test assuming equal variances: $P = 0.063$ [one-sided], $P = 0.126$ [two-sided]).
Conclusions: Binding estimates of ORI to huSER and moSER using arithmetic MIC methodology demonstrates a small but significant difference between the species. Conversely, AUIC analysis of time kill data suggested that ORI binding to huSER was similar to binding to moSER. These two methods provide the first cross-species examination of ORI serum protein binding and serve as a benchmark in situations where nonspecific drug binding precludes use of standard serum protein binding methods.

P1855 In vitro time-kill studies of oritavancin against clinical isolates of *Streptococcus pyogenes*

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Objectives: Oritavancin (ORI), a semi synthetic lipoglycopeptide, exerts bactericidal activity against drug-resistant Gram-positive bacteria. To characterise ORI activity in vitro we performed time-kill (TK) experiments against recent clinical isolates of *S. pyogenes*, including erythromycin (ERY)-resistant isolates.
Methods: 11 strains of *S. pyogenes* were tested in TK assays based on CLSI guidelines. To insure quantitative recovery of ORI, assays included 0.002% polysorbate-80 throughout. ORI and commonly recommended antibiotics for the treatment of *S. pyogenes* complicated skin and skin structure infections (penicillin G [PEN] and ERY) were tested alongside comparators vancomycin (VAN), teicoplanin (TEI), linezolid (LIN) and daptomycin (DAP) at fixed concentrations approximating their free peak (fCmax) and free trough levels in plasma when administered at standard doses taking into account serum protein binding. Cell counts were determined by serial dilution plating.
Results: ORI showed rapid concentration-dependent killing of all strains: when tested at its fCmax, ORI displayed bactericidal activity ($\geqslant$3 log kill

relative to starting inoculum) against ERY-susceptible *S. pyogenes* (n = 8) between 5 min to 3 h while for ERY-resistant strains (n = 3) ORI was bactericidal by between 30 min and 3 h. When tested at its free trough concentration, ORI demonstrated ⩾3 log kill within 5 h, regardless of ERY susceptibility status. Against all tested strains, ORI was significantly more rapidly bactericidal than VAN, ERY, PEN, TEI, LIN or DAP each at physiologically-relevant concentration.
Conclusions: The concentration-dependent killing of *S. pyogenes* by ORI in vitro was independent of ERY susceptibility status of the clinical isolates. These data support the conclusion that ORI displays rapid, concentration-dependent bactericidal activity against recent *S. pyogenes* isolates and is effective against drug-resistant isolates of *S. pyogenes*.

P1856 Comparative activity of oritavancin against recent genetically diverse methicillin-resistant *Staphylococcus aureus* isolates

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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).
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. Staphylococcal 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 MIC90 s were identical for the isolates regardless of the PVL type, SCCmec type or spa type. Based on MIC90 s, ORI was more potent than the comparators used in this study. ORI MIC90 s against all MRSA were 16-, 8-, and 4-fold lower than those for the LIN, DAP, and VAN, respectively.

Agent	MIC range (MIC_{90}), mg/L				
	Total (n = 58)	*PVL*+ (n = 33)	*PVL*– (n = 25)	SCC*mec*II (n = 16)	SCC*mec*IV (n = 42)
ORI	0.03–0.25 (0.25)	0.03–0.25 (0.25)	0.06–0.25 (0.25)	0.06–0.25 (0.25)	0.03–0.25 (0.25)
OXA	4–>64 (64)	4–64 (32)	8–>64 (>64)	32–>64 (>64)	4–>64 (32)
VAN	0.25–2 (1)	0.25–1 (1)	0.25–2 (2)	0.25–2 (2)	0.25–2 (1)
TEI	0.25–2 (1)	0.25–1 (1)	0.25–2 (2)	0.25–1 (1)	0.25–2 (1)
DAP	0.5–4 (2)	0.5–4 (1)	0.5–4 (2)	0.5–4 (2)	0.5–4 (1)
CIP	0.12–>64 (>64)	0.12–>64 (>64)	0.25–>64 (>64)	32–>64 (>64)	0.12–>64 (>64)
ERY	0.25–>64 (>64)	0.25–>64 (>64)	0.5–>64 (>64)	2–>64 (>64)	0.25–>64 (>64)
LIN	1–64 (4)	1–64 (4)	1–8 (4)	1–8 (4)	1–64 (4)

Conclusions: ORI MICs did not exceed 0.25 mg/L for any MRSA strain in this study. ORI activity was unaffected by presence of PVL, SCCmec type or spa type. Finally, MRSA isolates were more susceptible to ORI than to the comparators used in this study.

P1857 Telavancin disrupts bacterial membrane function by targeted interaction with the cell wall precursor Lipid II

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Objectives: Telavancin (TLV) is an investigational, bactericidal lipoglycopeptide antibiotic with potent activity against a broad range of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). The antibacterial mode of action of TLV includes inhibition of cell wall synthesis and disruption of essential membrane barrier functions. The purpose of this study was to further elucidate the mechanism by which TLV interacts with the bacterial membrane.
Methods: Bacterial membrane potential was assayed by flow cytometry using the fluorescent dye, DiOC_2(3), and early log phase *S. aureus* cells in cation-adjusted Mueller–Hinton broth. Cultures were incubated for 10 min in the pretreatment condition prior to exposure to TLV. Percent depolarisation was calculated relative to TLV-treated cells without pretreatment. Cultures that were suspended in nutrient-free medium (PBS with cations) were incubated for 10 min prior to TLV exposure.
Results: TLV (8 mcg/mL) induced complete depolarisation of *S. aureus* membranes. The role of Lipid II in TLV-induced depolarisation was demonstrated in bacteria expressing reduced levels of Lipid II. Pretreatment of *S. aureus* cells with inhibitors of Lipid II synthesis, fosfomycin, D-cycloserine, or bacitracin, suppressed TLV-induced depolarisation by 71%, 62%, and 73%, respectively. TLV-induced depolarisation was suppressed by 98% when Lipid II production was reduced by suspending cells in nutrient-free medium.
Conclusions: The membrane mechanism of TLV requires binding to the cell wall precursor, Lipid II. Therefore, through interaction with the membrane-embedded target Lipid II, TLV both inhibits peptidoglycan synthesis and disrupts membrane barrier function.

Epidemiology of MRSA

P1858 Prevalence of Panton-Valentine leukocidin genes in methicillin-resistant *Staphylococcus aureus* strains and susceptibility patterns

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Objectives: MRSA is an important cause of both nosocomial and community-acquired infections. Severe MRSA infections, including necrotising pneumonia, bacteraemia 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 cotrimoxazole, rifampin, vancomycin, teicoplanin, daptomycin, fusidic acid and linezolid. MIC90 for vancomycin, teicoplanin, daptomycin and linezolid were 2, 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 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 *Staphylococcus aureus* producing the Panton-Valentine leukocidin

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Objective: Studies comparing patients with methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *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 *S. aureus* susceptibility, clinical, microbiological, and outcome data regarding individual patients or group of patients with *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 *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 *Staphylococcus aureus* bacteraemia?

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Objectives: To determine if laboratory characteristics, such as susceptibility testing and molecular analysis, are associated with outcome in methicillin-resistant *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. Heteroresistance (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 ($\geqslant$10 days of bacteraemia), 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 (13.5%), 2.0 (0.5%). VAN MIC by Etest (n 189) was 1.0 (5.8%), 1.5 (75.7%), $\geqslant$2 (18.5%). VAN MIC by Vitek (n 189) was $\leqslant$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 5.3%.
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 $\leqslant$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 than 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 *Staphylococcus aureus* bacteraemia early in therapy: a classification and regression tree analysis

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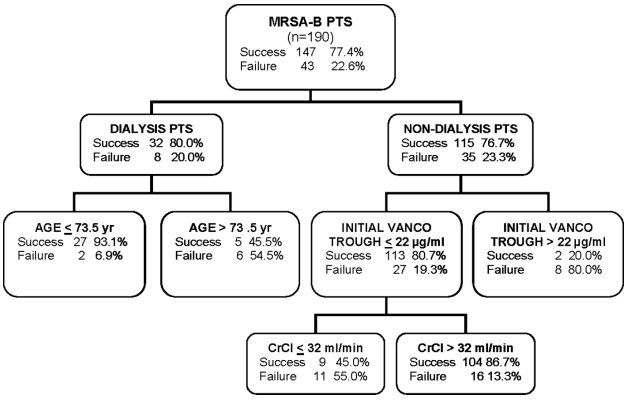
Objectives: To determine the effect of a combination of lab, patient, and treatment (tx) factors on outcome of MRSA bacteraemia (MRSA-B) treated with vancomycin (VAN).
Methods: We conducted medical records review and lab analysis of consecutive subjects/strains with MRSA-B treated with VAN. Failure was a composite of: 30-day mortality, microbiologic failure (positive cultures $\geqslant$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 55 y 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 (n 185) 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%), $\geqslant$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 3.4, 95% CI 1.3–9.1), cancer (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 dialysis subjects, the strongest predictor of failure was age with a cutoff of 73.5 y found by the analysis. In subjects with age >73.5 failure rate was 54.5%, whereas in subjects with age $\leqslant$73.5 failure rate was 6.9%. In non-dialysis 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 $\leqslant$22, the next best predictor of failure was creatinine clearance (CrCl). In subjects with $CrCl > 32$, failure rate was 13.3%. In subjects with $CrCl \leqslant 32$, 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.



P1862 How does persistent bacteraemia affect outcome in methicillin-resistant *Staphylococcus aureus* bacteraemia?

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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. Secondarily, 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 7days.

P1863 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

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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.

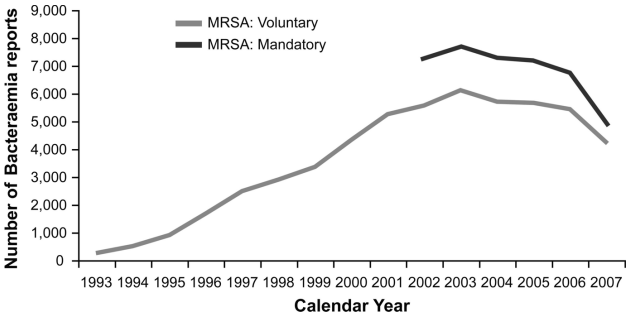
Methods: The analysis reported here covers MRSA bacteraemia cases reported on the basis of specimens collected during the period 2002 to 2007. National data from the Healthcare Associated Infection data capture system (HCAI DCS) was extracted on the basis of the total annual reports received.

Case reports sent to the voluntary LabBase system were extracted in August 2008. Reports in both systems are based on the specimen date, excluding duplicates or repeat specimens taken within two weeks of the first or previous report from the same patient.

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 HCAI 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.



P1864 Fitness cost: a possible explanation for the disappearance of multiresistant MRSA in Denmark during 1970–1975

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Objectives: Denmark and other European countries experienced the first epidemic of methicillin resistant *Staphylococcus aureus* (MRSA) during 1965–75, which was caused by an increasingly multi-resistant clone of the 83A phage com-plex. In Denmark it disappeared almost completely, being replaced by other phage types, predominantly only penicillin resistant. Albeit interventions including infection control measures and reduced administration of broad spectrum antibiotics were instituted, a solid explanation for the disappearance of the clone has not been found. The objective of this study was to investigate the fitness cost of

Staphylococcus aureus (*S. aureus*) isolates from the period 1957–1980 in correlation to resistance patterns.
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 phagetype (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; Pfts;< 0.0001); i.e. the growth rate was reduced with the more resistance markers the isolates carried. Furthermore, a significant negative correlation between fitness and time of isolation of PSTEM (penicillin, streptomycin, tetracycline, erythromycin and methicillin) resistant isolates in the period 1965–1975 was demonstrated (R2=0.70; 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 resistant isolates carried tet(M) 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.

P1865 Prevalence of *Staphylococcus aureus* in health professionals in a Brazilian teaching hospital

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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 *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 anaesthesical 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 sensibility 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 is also 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.

P1866 Trends in the incidence of methicillin-resistant *Staphylococcus aureus* nosocomial bloodstream infections in a tertiary care hospital in Greece: a three-year study

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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. Staphylococcal bacteraemia 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; Pfts;< 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.

P1867 The prevalence of carriers of methicillin-resistant *Staphylococcus aureus* in Copenhagen

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Objectives: The aim of the study was to elucidate the frequency of MRSA carriage in patients receiving treatment at the School of Dentistry in order to achieve a greater knowledge of the carrier state of MRSA in a population of children and adults in Copenhagen. Furthermore we wanted to investigate whether dentists and dental students were at risk of being carriers of MRSA.
Methods: During a period of two years from 01.10.06 to 01.10.08 2230 specimens from 1115 patients and 534 specimens from 267 dentists/dental students were collected. A specimen from both nostrils and a specimen from both tonsils were taken from each patient, i.e. two specimens per patient. Each specimen was immediately plated on a 5% blood agar and inoculated into a nutrient selective broth. Both were incubated overnight at 37ºC. At day two material from the broth was plated on a chrome agar plate and incubated overnight at 37ºC. Susceptibility testing was performed at day three using a 10 microgram cefoxitin disk (Oxoid, Basingstoke, UK) and semi-confluent growth on Iso-sensitest agar and incubated overnight at 37ºC. Resistance was

defined as ⩽21 mm (SRGA). Cefoxitin resistant *S. aureus* were sent to the Staphylococcus Laboratory at Statens Serum Institut to PCR for the mecA gene, spa typing and susceptibility testing.
Results: 1025 adults ⩾18 years with a mean age of 42.1 and a range of 18 to 91 years, 90 children ⩾2 years with a mean age of 7.5 and a range of 2 to16 years and 267 dentists and dental students with a mean age of 29 and a range of 18 to 71 years were included in the study. Two MRSA positive patients were found, one child and one adult. The child was positive for MRSA in the nose, the strain was a t022 spa type. It was a healthy 13 year old boy born in Denmark of Danish parents. He had not been hospitalised in Denmark or abroad and he had not been travelling abroad for the last year. The adult was also positive for MRSA in the nose, the strain was a t008 spa type. It was a healthy 27 year old Danish man who had been travelling abroad for 11 months the last year visiting South and Central America and was treated for a wound infection in a private clinic. None of the dentists/dental students were MRSA positive.
Conclusion: The prevalence of carriers of MRSA in Copenhagen seems to be very low and in this study only 0.2%. During the last two years the number of patients with MRSA in Denmark has declined as a result of the implementation of national guidelines.

P1868 Emergence of Panton-Valentine leukocidin-positive community-acquired MRSA and MSSA infections in an Italian hospital

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Objectives: The worldwide 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 associated with severe soft tissue infection and necrotising pneumonia. In this report the Authors describe 3 cases of PVL+CA-MRSA and 1 case of PVL+CA-MSSA infections observed in 2008.
Methods: *Staphylococcus aureus* (Sa) isolates in Mantova hospital (530 beds) were identified by conventional tests, followed by the determination of MIC for Oxacillin by the agar dilution method. The presence of lukS and lukF genes (encoding PVL) were defined by PCRs at the National Institute of Health (Istituto Superiore di Sanità) in Rome.
Results: A total of 348 *S. aureus* isolates were collected from different patients during 2008. Among them 97 (27.9%) were MRSA and 251 (72.1%) were MSSA. Three MRSA cases were PVL+ and also 1 MSSA case was PVL+. 2/3 cases of CA-MRSA were associated with skin and soft tissue infection and 1/3 case was sepsis complicated by meningitis and brain abscess. The case of PVL+CA-MSSA was associated to the cervical spondylodiscitis. Every patient was positive for Sa oro-nasal carriage. In just one case of PVL+ CA-MRSA there was a recent history of hospitalisation. No case of staphylococcal infection was reported among the relatives or close social contacts. The cases were resolved thanks to a treatment with glycopeptides, but one case required linezolid therapy. Mupirocin was used to eliminate Sa nasal carriage.
Conclusions: Specific surveillance of MRSA and MSSA infections in the community is required to monitor and prevent the spread of these PVL+ strains. The alert system and the control of the local epidemiology of PVL+ CA-MRSA/MSSA represents a priority even in small hospitals.

P1869 Surveillance of methicillin-resistant *Staphylococcus aureus* within Irish intensive care units

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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 Health Protection Surveillance Centre (HPSC) co-ordinates 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/coronary 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 0.27%) 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 case mix, size and 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.

P1870 Community-onset versus nosocomial bloodstream infections due to methicillin-resistant *Staphylococcus aureus* in Spain

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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 relation 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

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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 every year. 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 analyzed. 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).

Table 1. MRSA incidence densities (ID) (MRSA case-patients per 1,000 patient days) of 169 hospitals grouped into 5 regions

MRSA data	East	South-East	South-West	North	West	Total	p-value
ID (pooled mean)	0.96	1.37	0.78	0.79	1.00	0.98	
95% CI	0.94; 0.98	1.34; 1.40	0.78; 0.80	0.78; 0.81	0.98; 1.03	0.97; 0.99	
ID (median)	0.69	1.11	0.77	0.63	0.96	0.83	0.009

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.

P1872 Prevalence of MRSA and staphylococcal toxins among cystic fibrosis adult patients

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Objectives: *Staphylococcus aureus* which expresses numerous toxins to evade host defence is recognized as a major pathogen in Cystic Fibrosis (CF) patients. This study was designed to determine 1) the prevalence of MRSA and 2) the presence of toxins genes among *S. aureus* isolated from adult CF patients attending our CF unit.
Methods: Antibiotic susceptibilities were performed by the automated Wider system and the Kirby Bauer method according to CLSI recommendations. Methicillin resistance was confirmed by the PB2a detection (slidex BioMerieux). The mecA gene (encoding PBP2a), lukS and lukF genes (encoding PVL), tst (encoding toxic shock syndrome toxin), sem/seg (genes of the enterotoxin gene cluster egc) were defined by PCRs with specific primers.
Results: A total of 87 *S. aureus* strains were recovered out of 150 CF patients. Most of them (74/87) were MSSA. Among the 13 MRSA isolates (prevalence 8.6%) tested for the presence of genes encoding toxins, one was found to be positive for PVL, one for TST-1 and two for enterotoxins. On the other hand, among ten, randomly collected 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 emergency 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.

P1873 Epidemiology of MRSA bacteraemia and clinical relevance of reduced susceptibility to vancomycin

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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 $\geqslant$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 hVISAs. 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 ($p = 1$), relapse rate ($p = 0.48$) or rate of other complications ($p = 0.56$). SCCmec type IVh (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?

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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

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 in this study, 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.

P1875 Risk factors for methicillin-resistant *Staphylococcus aureus*

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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 *S. aureus* positive patients (only first isolate) and susceptibility testing result for oxacillin were retained. Logistic regression was used to identify risk factors for oxacilline 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. JO1C) 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 (95% CI) 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 chinolones (1.017–1.026); cephalosporins, monobactams and carbapenems (1.004–1.012); penicillins, aminopenicillins +/– clavulanic acid, β-lactamase stable penicillins (1.0006–1.0039).
Conclusions: These data strongly support the existence of a risk for acquired antimicrobial resistance in the major bacterial pathogen *S. 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.

P1876 Resident- and institution-related factors associated with methicillin-resistant *Staphylococcus aureus* carriage in nursing homes in western Switzerland

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Background: Nursing home (NH) environment is likely propitious to circulation of MRSA among residents and between NHs and acute care hospitals through transfers of residents. However, few data exist on the prevalence of MRSA carriage in NHs residents although this should be the basis for decisions on infection control measures.
Objectives: Assessment of the prevalence of MRSA carriage among residents of NH in Canton of Vaud (Western Switzerland), an area of presumably low MRSA prevalence (12% of *S. aureus* isolates in the local tertiary hospital), and identification of resident- and NH-related risk factors for carriage.
Methods: Point prevalence survey in residents from 130 of 155 NHs in Canton of Vaud. The proportion of residents randomly assigned to screening of MRSA carriage was 25%, 33% or 50% in NH of >100, 50 to 99, and <50 beds, respectively. Nose, groin and wounds of included residents were swabbed, and an urine culture was obtained in the presence of a urinary catheter. A short questionnaire was completed for each included resident and each NH.
Results: *Residents*: 273 of 2275 (12%) screened residents were MRSA carriers. Independent risk factors for carriage were diabetes mellitus ($p = 0.015$), wound ($p = 0.041$), urinary catheter ($p < 0.001$), hospital stay within the previous 2 years ($p = 0.013$) and antibiotic therapy within the previous 4 weeks ($p = 0.001$).
Nursing homes: MRSA-positive residents were identified in 81 of 130 (62%) NH, with a prevalence that ranged from 0 to 57% (median 7%). NHs with MRSA carriers were larger ($p = 0.002$), had less individual rooms ($p = 0.013$), and had a trend toward a lower nurses-to-residents ratio ($p = 0.069$).
Conclusions: Prevalence of MRSA carriage in NH of Western Switzerland varies from 0 to 57%. This is influenced by structural characteristics of NH in addition to classical resident-related risk factors. These results highlight the need to reassess control strategies in NH. Further studies are needed to determine the potential health benefits of reducing MRSA colonisation rates in this setting.

P1877 5-year evolution of methicillin-resistant *Staphylococcus aureus* carriage in nursing-home residents of western Switzerland

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Background: Nursing homes (NH) environment is probably favourable to the acquisition and spread of MRSA. In addition, transfers of residents to or from acute care hospitals are opportunities for circulation of MRSA. It is therefore important to determine to what extend enforced infection control measures in NHs appropriately control the spread of MRSA in this setting.
Objectives: To measure the evolution of MRSA carriage over 5 years among residents of NHs in Canton of Vaud (Western Switzerland), an area of presumably low MRSA prevalence (12% of *S. aureus* isolates in the local tertiary hospital).
Methods: Point-prevalence surveys within the 155 NHs in Canton of Vaud in 2003 (17 NHs), 2005 (34 NHs), and 2008 (130 NHs). All NH's residents were screened for MRSA carriage during 2003's and 2005's surveys. In 2008 the proportion of residents randomly assigned to screening of MRSA carriage was 25%, 33% or 50% in NHs of >100, 50 to 99, and <50 beds, respectively. Nose, groin, and wounds of included

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 NH'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.
355 MRSA-positive residents had a median 12-month (range: 3–24) follow-up of MRSA infection. 57 infections occurred in 44 residents, which represented an incidence rate of 0.1 episode per resident-year. The most frequent sites of infection were wounds (43%) and the urinary tract (32%).
Conclusions: We observed a 167% increase (up to 12%) in prevalence of MRSA carriage over 5 years in NH'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.

P1878 *Staphylococcus aureus* colonisation/infection in a neonatal intensive care unit: a four-year study

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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-sulfamethoxazole (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 54days, 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 14days (range 3–207d). 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 MSSA was identified in 29 cases (bacteraemia, 3; UTI, 2; opthalmia, 18; cutaneous infections, 3; umbilicitis, 3), and to MRSA in 17 cases (ophthalmia, 8; cutaneous infections, 7; umbilicitis, 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

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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 $\geqslant 4\,\mu g$ for oxacillin were identified as MRSA. Intrinsic high level resistance (Mec A positive) and borderline oxacillin resistant staphylococcus aureus (BORSA) were detected by amoxicillin-clavulanate E-Test strips. Strains with MIC of $\geqslant 4\,\mu g$ for oxacillin and $\geqslant 8\,\mu g$ for amoxicillin-clavulanate were identified as mec A positive MRSA. Other staphylococcus with MIC $\geqslant 4\,\mu g$ for oxacillin and $\leqslant 4$ for amoxicillin-clavulanate were identified as mec A negative MRSA (BORSA). 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%) MRSA were Mec A positive and 23out of 90(25.5%) were mec A negative (BORSA). Although most of the MRSA strains were isolated from surgical site infections, there were no statistically significant difference between types of staphylococcus aureus growing from variant sites of infections. Only one (1.49) of the Mec A positive MRSA had reduced susceptibility to vancomycin but all of the mecA-negative MRSA (BORSA) were sensitive to it.
Conclusion: Because one fourth of our staphylococcus strains are mec A negative BORSA 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

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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

2005. As a consequence, β-lactam derivatives proved an in vitro activity of 46.7–54.1% for co-amoxiclav, 46.6–54.1% for cefotaxime, and only 7.3–11.2% for penicillin. Among other tested molecules, clindamycin reached a comprehensive 50% susceptibility rate (40.8–54.3% of all tested strains), followed by erythromycin (40.3–54.7%), and gentamicin (42.6–49–5%). No statistically significant temporal variations of antimicrobial susceptibility rates occurred during the four-year study time

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. Despite a stable, significant rate of methicillin resistance rates (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.

P1881 Assessment of screening all patients and the environment for MRSA in an acute hospital

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Objectives: MRSA is endemic in Irish hospitals. A four-year research grant was awarded to study the prevalence of MRSA in new patient admissions to an acute tertiary referral hospital, prior to assessing the value of enhanced decontamination, improved hand hygiene compliance and molecular methods for the rapid detection of MRSA. We sought to establish baseline MRSA prevalence data over a 12-month period prior to the implementation of the interventions.

Methods: All consenting patients admitted to two medical and two surgical wards of a 700-bed acute hospital were screened within 72 hours of admission ('admission' patients) and at weekly intervals over a six-week period on each ward. In-patients hospitalised for longer than 72 hours were also screened to establish the extent of the MRSA reservoir in these wards ('reservoir' patients). Sampling was also undertaken to investigate the MRSA burden in the inanimate environment.

Results: MRSA was isolated from 10% (92/938) of all patients, including, 8% (51/635) of 'admission' and 13% (41/303) of 'reservoir' patients. Of the 635 'admission' patients, MRSA was recovered from 5% of patients (32/635) on admission; 2% of MRSA-positive patients were already known to be MRSA-positive and 2% (4/635) were considered to be hospital-acquired. Of the 303 'reservoir' patients, MRSA was isolated from 7% of patients (22/303) who were hospitalised for longer than 72 hours. Seven MRSA-positive patients (2%; 7/303) were known to have had MRSA on admission but MRSA was recovered from 12 patients (4%; 12/303) with previous MRSA-negative screening results. Six per cent (77/1361) of environmental screens including; mattresses (8%; 36/462), bed frames (5%; 5/106), patient lockers (3%; 4/125), air sampling (12%; 21/180) were MRSA-positive.

Conclusions: Screening revealed a high prevalence of MRSA among patients hospitalised for over 72 hours, and to a lesser extent amongst 'admission' patients. These results indicate that additional efforts are required to reduce patient and environmental contamination with MRSA.

P1882 An anonymous survey to determine the nasal colonisation rates of methicillin-resistant *Staphylococcus aureus* in patients attending the emergency department of a tertiary referral hospital in Dublin, Ireland

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Objectives: In recent years, in North America and continental Europe, rates of nasal colonisation and severe infection caused by meticillin-resistant *Staphylococcus aureus* (MRSA) have been increasing, in particular community-acquired strains (CA-MRSA). The aim of this study was to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonisation among patients presenting to the Emergency Department of a large urban tertiary referral hospital in Dublin.

Methods: 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, living 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 ($p \leqslant 0.05$). 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 ($p \leqslant 0.01$) 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.

P1883 Occurrence of methicillin-resistant *Staphylococcus aureus* among clinical samples in Tehran and its correlation with the site of infection

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Introduction and Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for an increasing number of serious nosocomial and community acquired infections including superficial lesions and wound infections, osteomyelitis, endocardiris, pneumonia, bacteraemia, Toxic shock syndrome and food poisoning. The purpose of this study was to define the prevalence of of MRSA strains among *S. aureus* strains isolated from selected Tehran hospitals with conventional and molecular methods.

Material and Methods: A total 235 isolates were evaluated by disk diffusion and MIC agar dilution tests according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) and PCR assay for mecA gene.

Table 1. Resistance against oxacillin in different specimens with various phenotypic and genotypic tests

Sample	Resistant		
	MIC*	Disk diffusion	*mecA* gene positive
Blood (n = 60)	29 (48%)	30 (50%)	30 (50%)
Urine (n = 37	16 (43%)	16 (43%)	17 (46%)
Cutaneous sample (n = 43)	18 (42%)	18 (42%)	19 (44%)
Respiratory tract (n = 55)	27 (49%)	27 (49%)	28 (51%)
Other (n = 9)	4 (45%)	5 (46%)	5 (46%)
Nasal swab (n = 31)	11 (35%)	12 (39%)	11 (35%)

*According to NCCLS guidelines isolates with MIC $\geqslant 4\,\mu g$ are resistant to oxacillin.

Results: from 2006 to 2007 a total of 235 strains from patients and healthy persons (163(%69) men and 72(%31) women) were evaluated. Our strains isolated from blood, urine, coetaneous samples, respiratory tract samples, nasal swaps and miscellaneous samples. Prevalence of MRSA strains in different samples is shown in table 1.
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 Exudates or Bone marrow) (%45), Urine (%43), Coetaneous samples (%41) and finally Nasal swabs (%34). These differences were not statistically significant ($P > 0.05$).

P1884 Antimicrobial susceptibility in PVL positives *S. aureus* strains. 2005–2008 study

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Objectives: Panton-Valentine leukocidin (PVL)-producing *Staphylococcus aureus* is emerging as a serious problem worldwide. It has been described as usually causing skin and soft-tissue infections, but also necrotising pneumonia affecting previously healthy young people and associated with a very high mortality.
Therapeutical management in these infections has been long debated without any treatment consensus. Clindamycin and Daptomycin have been proposed as effective treatments in patients with soft tissue or skin infections.
In the presented study we investigated the activity of Daptomycin and others antistaphylococcal agents against such isolates.
Methods: A retrospective study was performed in order to detect Staphylococcal strains harbouring PVL genes. 2014 *S. aureus* strains from our hospital (January 2005 – June 2008) were analyzed by an in-house Real-Time PCR assay, 45 isolates from 38 patients were positive. Identification and antimicrobial susceptibility testing were performed against 24 antimicrobial agents using the BD Phoenix™. Macrolide resistance was performed by using the disk induction test. Additional E-test susceptibility testing in Muller Hinton II Agar was performed for the new antimicrobial daptomycin.
Results: Samples origin was: 37 of the 45 (82.22%) from complicated skin or soft tissue infections (cSSTI), 3 (6.66%) from blood culture, 3 (6.66%) from Infection Control procedures, 1 (2.22%) from a infected surgical wound and 1 (2.22%) from a bronchial aspirate.
Number of strains and percentages of resistance against selected antimicrobials (OXA = oxacillin, ERY = erythromycin, CLI = clindamycin, GEN = gentamicin, CIP = ciprofloxacin, RIF = rifampicin, SXT = trimethoprim/sulfamethoxazole, VAN = vancomycin, DAP = daptomycin), are summarised in the table. No resistance to linezolid, vancomycin and daptomycin was found.
MICs to Clindamycin were $\leqslant 0.5$ in all the isolations but 7 of the strains (15.55%) showed a MLSB phenotype conferring a inducible clindamicyn resistance. Daptomycin MIC 50 was 0.24 mg/l and MIC 90 0.38 mg/l, 4 and 3 times higher respectively to the accepted susceptible breakpoint of 1 mg/l.
Conclusion: Clindamycin showed inducible resistance in more than 15% of the PVL positive *S. aureus* strains tested, Daptomycin was shown to be active against all of them. Daptomycin should be proposed to treat PVL cSSTI.

Table. Number of strains and percentages of resistance

Resistance	OXA	ERY	CLI	GEN	CIP	RIF	SXT	VAN	DAP
Number	29	18	7	0	9	1	1	0	0
Percentage	64.44	40	15.55	0	20	2.22	2.22	0	0

P1885 *Staphylococcus aureus* methicillin-resistant colonisation among healthcare workers at a general hospital

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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 t008-ST8-IV-agr 1 (CC8) (n = 2), and t018-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 (t008-ST8-IV-agr1) is a matter of concern. The other clone belongs to the international British EMRSA-16 (t018-ST36-II-agr 3) that is endemic in the studied hospital. HCWs could be the reservoir for the spread MRSA strains.

P1886 Methicillin-resistant *S. aureus* infections among patients in an emergency department

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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.

P1887 **Costs and benefits of a peri-operative screen-and-treat strategy in nasal carriers of *S. aureus***

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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.

P1888 **Carriage of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in medical microbiology laboratory personnel**

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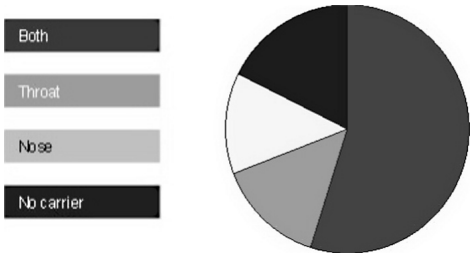
Objective: To determine whether personnel working in clinical microbiology laboratories in The Netherlands is at increased risk for colonisation with MRSA.
Methods: Anterior nares and throat swab samples were obtained from 266 employees of five different clinical microbiology laboratories. Both swabs were placed in enrichment broth (Becton, Dickinson and Company, France); these were incubated for 48 hours at 37ºC and then subcultured by plating aliquots on a chromogenic selective medium (bioMerieux, France) for 24 hours at 37ºC for the isolation of MRSA, and on a mannitol salt agar for 48 hours at 37ºC for the isolation of *S. aureus*. MRSA and *S. aureus* were identified according to standard protocols. 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 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 personel 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.



Staphylococcus aureus carriers among employees of five hospitals.

P1889 **Estimating the effect of methicillin-resistant *Staphylococcus aureus* infection on length of stay in an intensive care unit using a longitudinal model**

A. Barnett, R. Batra, J. Edgeworth, N. Graves, J. Robotham, B. Cooper (Brisbane, AU; London, UK)*

Objectives: To quantify increased length of stay in the intensive care unit (ICU) and ICU mortality due to methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: We use a multinomial longitudinal model for estimating the daily probability of death and discharge applied to more than five years of detailed longitudinal ICU data. We then extend the basic model to estimate how the effect of MRSA infection on the probability of discharge changes over time, and to quantify the number of excess ICU days due to infection as a function of the day of the ICU stay on which the infection was acquired.
Results: We find that MRSA infection decreases the relative risk of discharge (relative risk ratio 0.76, 95% credible interval: 0.62, 0.93) but is only indirectly associated with increased mortality. An infection on the first day of admission resulted in a mean extra stay of 0.6 days for a patient with an APACHE II score of 10, and 1.2 days for a patient with an APACHE II score of 30. The decrease in the relative risk of discharge remained fairly constant with day of MRSA infection, but was slightly stronger closer to the infection time.
Conclusions: These results confirm the importance of MRSA infection in increasing ICU stay, but highlight the attributable length of stay due to MRSA can be highly dependent on whether an infection is acquired early or late in a patient's stay. The results also suggest that previous work may have systematically overestimated the effect size.

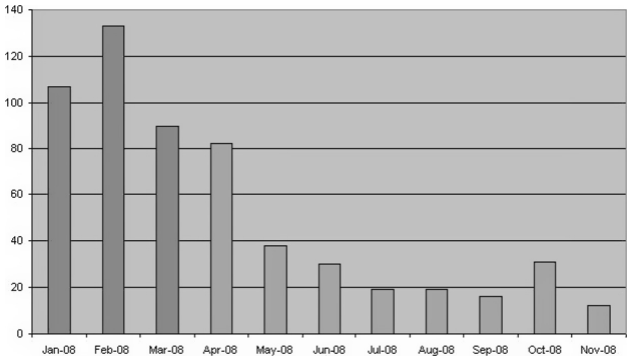
MRSA: screening and IC measures

P1890 **Exploring the fourth dimension: the clinico-economic impact of a distinct model of MRSA screening by PCR in United Kingdom**

A. Guleri, R. Palmer, C. Danson, 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.



Reduction in total MRSA infection episodes – PCR introduced mid March.

P1891 Molecular assay for the rapid screening of methicillin-resistant *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 *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. Nighty-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.

P1892 Cost analysis of rapid diagnostic testing of methicillin-resistant *Staphylococcus aureus* using PCR or chromogenic agar in a low endemic setting

M. Wassenberg, A. de Wit, B. van der Zanden, M. Bonten (Utrecht, Bilthoven, NL)*

Objectives: To determine whether rapid diagnostic testing of methicillin-resistant *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 GeneOhmTM 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 haven 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.

P1893 Evaluation of the impact of screening and signalling carriers of methicillin-resistant *Staphylococcus aureus* on hand hygiene compliance and MRSA cross-transmission in 4 intensive care units

H. Hitoto, A. Kouatchet, L. Dubé, A. Mercat, M.L. Joly-Guillou, M. Eveillard (Angers, FR)*

Objective: Strategies combining systematic screening of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers and subsequent implementation of barrier measures are usually recommended, but are

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 HHC 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 (CI95%= (1.97–2.03)) vs. 0.70 (CI95%=(0.68–0.72). I2 was higher in P1 (16.0 (CI95%= (14.0–18.0)) than in P2 (11.4 (CI95%=(7.4–15.4)).
Conclusion: We failed to identify any advantage by using screening and signalling MRSA carriers in those 4 ICU.

P1894 Costs of nosocomial methicillin-resistant *Staphylococcus aureus* pneumonia

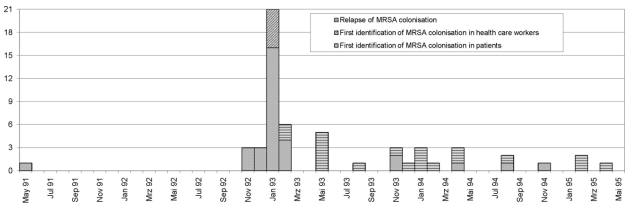
E. Ott, K. Graf, F. Schwab, C. Reichardt, I.F. Chaberny (Hanover, Berlin, DE)*

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€; $p = 0.011$). 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.

P1895 Eradication of an epidemic methicillin-resistance *Staphylococcus aureus* from a geriatric university hospital. Evidence from a >10-year follow-up

D. Mertz, R. Frei, N. Periat, C. Scheidegger, M. Battegay, W. Seiler, A.F. Widmer (Basel, CH)*

Objectives: Published studies of successful eradication of methicillin-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.



P1896 Development and use of an on-line tool for completion of MRSA bacteraemia root cause analysis

K. Hardy, S. Gossain, A. Penny (Birmingham, UK)*

Objectives: To develop an on-line tool that allows timely and targeted completion of a root cause analysis investigation (RCA) for MRSA bacteraemia cases within an acute hospital setting.
Methods: For all MRSA bacteraemia cases in England there is a requirement to complete a RCA. The process in our Trust involved completion of a paper form which was sent back to a central point for analysis. This resulted in partially completed forms, with varied data being provided. Completion and data entry for analysis were time consuming and the missing data meant the analysis was not standardised

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 RCA process to be completed electronically. Initially the electronic 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. Messaritaki, E. Bolikas, C. Tsioutis, 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 administrated 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 $\geqslant 1$ (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.

Procedure specific SSI rates and risk-adjusted comparisons with international data

NNIS Operative Category	Study results		US rates[1]		Spanish rates[2]		UK rates[3]	
	No. of SSI/ procedures	SSI rate (%)	SIR	p	SIR	p	SIR	p
Open reduction of fracture	26/456	5.7	5.0	<0.001	2.0	0.001	1.9	0.002
Hip prosthesis	19/506	3.8	3.0	<0.001	1.0	0.531	2.0	0.003
Knee prosthesis	14/268	5.2	4.7	<0.001	2.6	0.001	1.3	0.217
Spinal fusion	9/248	3.6	1.7	0.080	0.9	0.459	–	–

NNIS, National Nosocomial Infection Surveillance system; SSI, surgical site infection; SIR, standardised infection ratio.
[1] Am J Infect Control 2004;32:470–85. [2] Am J Infect Control 2006;34:134–41. [3] UK Health Protection Agency 2006.

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, the 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% 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 statistical 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.

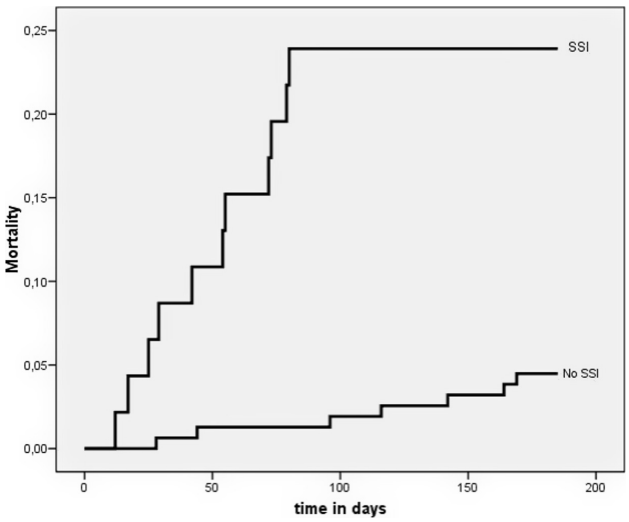
P1899 Consequences of surgical site infections after colorectal surgery

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

versus 0.3 days, $p < 0.001$). Also, the mortality in infected patients was significantly higher (see figure, $p < 0.001$).

Conclusions: The incidence of SSI of patients undergoing colorectal surgery is high (20%) and is associated with significant mortality and prolongation of stay on the ICU and in the hospital.



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, in 2007 the GIS-INCARD documented a rate of SWI acceptably low. Aim of the present study was to verify the incidence of SWI and to re-evaluate the adherence to standard recommandations 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, intra and post-operative risk factors, Staph. aureus nasal colonisation, timing and modality of depilation 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 hospitalisation was shorter (−0.5 days), less patients had preoperative hyperglycaemia (−5%), more patients were investigated for staphylococcal nasal carriage (+8%), less patients had hair shaving (−4%) and more patients had hair removal soon before surgery (+17%). According to scientific recommendations, the use of antibiotic prophylaxis regimens based on glycopeptides cut in half (−8%), while increased the number of patients receiving a single dose of vancomycin as adjuvant (+12%).

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.

	2005–2006	2008
Incidence of sternal wound infection	2.63	2.62
Mean age (years)	65.9	68.8
Mean preoperative hospitalisation (days)	5.54	5.09
Coronary artery bypass graft/valve replacement (%)	58.4/34.9	61.6/29.6
Elective procedures (%)	88.9	84.2
Cardiopulmonary bypass (%)	90.9	95.1
Length of surgery >5 hours (%)	22.3	24.4
Preoperative hyperglycaemia (%)	22.7	17.8
Cigarette smoking (%)	21.3	20.3
Patients with cultures for staphylococcal nasal colonisation (%)	52.2	60.5
Staphylococcal nasal carrier (% of cultured patients)	8.6	8.1
Shaving of operative site (%)	97.8	93.9
Shaving of operative site at day of surgery	40.6	57.2
Prohhylaxis with 1st–2nd generation cephalosporins (%)	75.4	78.8
Prophylaxis with glycopeptides – multiple doses (%)	16.4	8.4
Prophylaxis with glycopeptides – single doses (%)	8.1	20.9
Prophylaxis with not antistaphylococcal agents (%)	2.3	5.6

P1901 Risk factors for fungal surgical site infections in recipients of ventricular-assist devices

A. Schweiger, C. Ruef, 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 under 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 mediastinum (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 $\geqslant$14 days (3/4 in infection group, 3/8 in controls) was significantly associated with development of candidal infection ($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 $\geqslant$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.

Case	Organism	Positive fungal cultures: No. of body sites	Days prior to infection	Blood cultures	Type of infection	Onset of infection: Day after admission	VAD-day	Treatment: Drug	Duration	Other nosocomial infections	Outcome	Death related to fungal infection
1	*Candida albicans*	1 (dialysis catheter)	1	Neg	Mediastinitis (SSI-MED)	19	6	Caspofungin Fluconazole	6 d 82 d	BSI-LCBI, CVS-VASC, SSI-VASC, SSI-IAB	Transplantation on day 94. Exitus day 1 post-transplantation (haemorrhage and thrombosis of left ventricle)	?
2	*Candida albicans*	4 (urine, respiratory, abdominal wound, genital)	12	Pos	Mediastinitis (SSI-MED)	21	2	Fluconazole Caspofungin	10 d 61 d	SSI-IAB, PNEU-PNU1, UTI-ASB, SSI-SKIN	Removal of VAD after 49 d of support. Exitus due to haemorrhage on day 17 after removal	No
3	*Candida parapsilosis*	1 (respiratory)	3	Neg	Soft tissue (SSI-ST)	52	28	Caspofungin Fluconazole	16 d 40 d	SSI-MED, CVS-VASC, SSI-IAB	Death on day 89 under support due to aspiration-Pneumonia	No
4	*Candida albicans*	4 (inguinal wound, urine, respiratory, VAD)	189	Neg	Soft tissue (SSI-ST)	223	195	Fluconazole	81 d	BSI-LCBI, LRI-BRON, CVS-VASC, UTI-ASB, PNEU-PNU1	Transplantation. Death 2 years after transplantation	No

Neg, negative; Pos, positive.
BSI-LCBI: Laboratory-confirmed bloodstream infection; CVS-VASC: Venous infection; LRI-BRON: Bronchitis without evidence of pneumonia; PNEU-PNU1, Pneumonia; SSI-IAB: Surgical site infection of abdominal space; SSI-MED: Surgical site infection of mediastinum; SSI-SKIN: Surgical site infection of superficial incisional site; SSI-VASC: Surgical site infection of blood vessels; UTI-ASB: Asymptomatic bacteriuria.

P1902 Associations between operative site microbial counts and procedure classification in neurosurgery

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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, clean 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% preprep, 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

P1903 Post-discharge surveillance of surgical site infections after total hip and knee arthroplasty

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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 2.26% 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.

P1904 Reducing surgical site infection rates in cardiac surgery: results of 10-year infection control programme

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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 2.22, 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

0.75% (p = 0.25). The adjusted odds ratios (OR) for these infections at the end of 2002 compared to December 31, 1998, were as follow: SUP 0.5 (95% confidence interval [CI-95], 0.26–0.97, OS 0.44 (CI-95, 0.39–2.08), MED 0.09 (CI-95, 0.012–0.73), and MRSA 0.49 (CI-95, 0.15–1.64, respectively. The 1-year mortality rate among patients with SSIs was 8.3% as compared with a mortality rate of 4.6% among non-infected patients. By logistic regression analysis, SSI remained an independent risk associated with an increased mortality (OR 1.8; CI-95, 1.12–2.93).
Conclusions: We observed significant reductions in infection rates in most types of infections, particularly in superficial SSI and mediastinitis. These differences remained significant when adjusted for potential confounding variables.

P1905 Evaluation of three preoperative preparation products when used in a preoperative site-wash regimen

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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 preparation (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 microorganisms produced at a specific site, the knee, by applications of a marketed PreOp Prep product over the course of 4 consecutive days. Three different commercially available products were tested – TRISEPTIN® Water-Aided, MaxiClens®, 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 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 in 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).

Sample time (pre/post-application)	$\log_{10}$ differences from day 1 pre-application population					
	TRISEPTIN® Water-Aided (70% EtOH v/v) N = 10 Day 1 Pre-Application (Baseline) Baseline Mean = 2.56 (S.D. = 0.64)		CHLORAPREP® (2.0% w/v CHG in 70% w/v IPA) N = 10 Day 1 Pre-Application (Baseline) Baseline Mean = 2.62 (S.D. = 0.60)		MAXICLENS™ Antiseptic Skin Cleanser (4% w/v CHG) N = 10 Day 1 Pre-Application (Baseline) Baseline Mean = 2.43 (S.D. = 0.52)	
	Mean Reduction (SD)	95% CI	Mean Reduction (SD)	95% CI	Mean Reduction (SD)	95% CI
Post-Day 1	2.24 (0.72)	1.73, 2.75	2.45 (0.61)	2.01, 2.89	2.20 (0.47)	1.86, 2.54
Pre-Day 2	0.58 (0.92)	−0.08, 1.24	0.21 (0.27)	0.02, 0.40	0.33 (0.57)	−0.08, 0.73
Post-Day 2	2.52 (0.65)	2.05, 2.98	2.51 (0.55)	2.11, 2.90	1.80 (1.07)	1.03, 2.56
Pre-Day 3	0.81 (0.87)	0.19, 1.43	0.41 (0.49)	0.07, 0.76	0.61 (0.78)	0.05, 1.17
Post-Day 3	2.50 (0.71)	1.99, 3.01	2.47 (0.66)	2.00, 2.94	2.32 (0.48)	1.98, 2.67
Pre-Day 4	0.75 (0.86)	0.14, 1.36	0.54 (0.33)	0.31, 0.78	0.81 (0.66)	0.34, 1.28
Post-Day 4	2.56 (0.64)	2.11, 3.02	2.61 (0.60)	2.18, 3.04	2.43 (0.52)	2.05, 2.80

S.D. = Standard Deviation; CI = Student's *t* Confidence Interval, α = 0.05.

Conclusion: A PreOp SW regimen using any of the products tested – TRISEPTIN® Water-Aided, MaxiClens®, or ChloraPrep® – plainly would present a much reduced population of microbial flora in challenge to the site PreOp Prep procedure to be performed immediately prior to surgery. The data indicate that the PreOp SW process should be initiated at least 2 days prior to the scheduled surgery, and that 3 or 4 days prior would be better yet.

P1906 Efficacy of chlorhexidine gluconate against *Staphylococcus aureus* in a respiratory epithelial infection model

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Background: *Staphylococcus aureus*, a significant pathogen, can invade via skin or mucosal epithelium. Chlorhexidine gluconate (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 1×10^6 CFU/mL for 15 min prior to treatment with CHG (2.0–128 ug/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* were 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 ug/mL; and cell associated was achieved at CHG ⩾ 128 ug/mL.

Epithelial cell line	Chlorhexidine gluconate		
	S. aureus ⩾2 $\log_{10}$ CFU/mL kill supernatant	*S. aureus* ⩾2 $\log_{10}$ CFU/mL kill epithelial cell-associated	Epithelial cytotoxicity (LD50)
Nasal (RPMI 2650)	⩾2 ug/mL (0.0002%)	⩾16 ug/mL (0.0016%)	⩾64 ug/mL (0.0064%)
Lung (A549)	⩾4 ug/mL (0.0004%)	⩾16 ug/mL (0.0016%)	⩾128 ug/mL (0.0128%)

P1907 Economic aspects of deep sternal wound infections

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Objectives: 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 sings 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 the mean cost of CABG alone (19258.39 Euro, $p < 0.0001$) The average length of stay (LOS) in hospital was more than two times longer (34.4 days vs. 16.5 days) although the average LOS on ICU was shorter (2 days vs. 5.3 days).
Conclusion: DSWI is a devasting complication for surgeons and patients. But it is also an important economic factor for the hospital. Measures for the reduction of DSWI will be cost-effective. Therefore a bundle of infection control measures will be essential.

Diagnosis of viral infections

P1908 The combination of antibody bridging and avidity index methodology for reliable and simple determination of infection phase

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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 (ABAI) methodology for rapid in vitro serodiagnostic testing. We developed ABAI methods for two different fluorescence immunoassay platforms, the heterogeneous time-resolved fluoroimmunoassay (TR-FIA) 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 ABAI 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.

P1909 Novel multianalyte point-of-care test for pathogen-specific diagnosing of respiratory infections

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Objectives: Unfortunately, pathogen-specific diagnosis and treatment of respiratory infections is seldom possible. This is due to overlapping symptoms, long result-time of centralised laboratory testing, and the lack of efficient point-of-care diagnostic tests. Empirical treatment of respiratory infection patients has lead to overuse of antibiotics and inefficient use of virus-specific drugs. We have developed an immunoassay methodology which allows rapid and automated multianalyte detection of respiratory pathogens at the point-of-care.
Methods: The one-step immunoassay test is based on a separation-free ArcDia TPX fluorescence detection technology and dried reagents. This enables simple assay protocols; A swab sample from the nose or the throat is dissolved in sample buffer and subjected to automated quantitative multianalyte testing. The multianalyte test provides results within 20 minutes. The pathogen portfolio of the test system covers a significant proportion of respiratory infection cases, currently including:

- Group A streptococci
- *Streptococcus pneumoniae*
- influenza A and B viruses
- respiratory syncytial virus
- metapneumovirus
- adenovirus
- parainfluenza 1, 2 and 3 viruses.

Results: Validation of the methods with clinical samples ($n = 400$) and standard preparations showed similar analytical sensitivities (on average 90–95%) and specificities (on average 99.6%) to the reference laboratory methods. Cross-reactivities were not observed between analyte pathogens or microbes frequently present in the samples. The results demonstrate that the new methodology enables rapid multianalyte testing of respiratory pathogens. The test reports positive results in 20 minutes while the results for low positive and negative samples are reported in two hours.
Conclusion: Due to its simplicity and low cost structure, the methodology is well suited for rapid point-of-care testing, allowing accurate diagnosis and pathogen-specific medication. The test would bring the performance of standard laboratory methods into point-of-care testing. The use of such a rapid multianalyte test in clinical settings is expected to reduce the unnecessary use of antibiotics and to enable more efficient virus-specific medication.

P1910 Evaluation of the performance of RSV Respi-Strip® in comparison with cell culture and reverse transcriptase PCR

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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-Bioconcept), 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

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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 µL of each dilution and preserved respectively at room temperature or 4ºC 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 µL 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

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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 (Biotest) 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. 2×10^5 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 (Biotest 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 ($p=0.000355$ 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 antigenaemia HCMV assay.

P1913 Analytical performance of the new Access CMV IgG assay

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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

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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% (1194/1209). 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.

P1915 MRSA on a closed psychiatric ward

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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. Mupirocine nasal ointment was applied according to the common standard. 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 0 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 therapy. 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.

P1916 Diagnosis of CMV infection by serology: comparison of methods

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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 (Biomerieux); 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 44 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.

P1917 Performance evaluation of the Access CMV IgG assay performed on the UniCel DXC 880i in a French hospital

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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 LIAISON 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 LIAISON 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 (1.1%). 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 repetability 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 is performed shows many advantages related to organisation strategies 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 can be easily integrated and used in a laboratory.

P1918 Performance characteristics of an anti-Varicella zoster glycoprotein IgG ELISA

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Objective: To evaluate the performance of the VaccZyme™ anti-Varicella 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 $\geqslant$150 mIU/ml, 26% had antibody levels <100 mIU/ml and 11% were within the range of 100–150 mIU/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: See the table.

Performance characteristic	Anti-VZVgp IgG
Intra-assay reproducibility (%CV)	2.3–3.6
Inter-assay reproducibility (%CV)	1.5–9.7
Linearity R^2	>0.999
Linearity % recovery	97
Sensitivity relative to TRFIA (%)	98.5
Specificity relative to TRFIA (%)	97.7
Agreement relative to TRFIA (%)	98.3
Correlation coefficient (R^2)	0.81

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.

P1919 Comparison of Siemens IMMULITE® 2000 versus DiaSorin LIAISON® serology in case of Epstein-Barr virus

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Objective: Two new chemiluminescent enzyme immunoassays for EBV VCA IgM and EBNA IgG detection in serum on the IMMULITE® 2000 automated analyzer (Siemens Healthcare Diagnostics, Los Angeles, US) have been evaluated versus the immunoassays of the LIAISON® system (DiaSorin, Saluggia, Italy).
Methods: The IMMULITE® EBV performance and serological status agreement were compared to the LIAISON® EBV VCA IgM and EBNA IgG assays. For all samples with discordant results in interpretation of the EBV serological status, the samples were retested using the EUROLINE® EBV IgM and IgG blots (EUROIMMUN, Lübeck, Germany) as the gold standard.
Results: In case of 957 human serum samples EBNA IgG determined by IMMULITE® was compared to EBNA IgG determined by LIAISON®. Results indicated an agreement of 93% between the two systems. Comparison of IMMULITE® versus LIAISON® EBV VCA IgM resulted in a 91% agreement.
811 of the 957 serum samples (85%) tested by IMMULITE® and LIAISON® demonstrated concordant results for VCA IgM and/or EBNA IgG, therewith showing 100% agreement for the interpretation of the EBV serological status.
The 146 remaining serum samples tested by IMMULITE® and LIAISON® showed discordant results for VCA IgM and/or EBNA IgG. However, in case of 79 of these samples the interpretation of the EBV serological status was similar (54% agreement). Of the remaining 67 discordant results, 66 samples were additionally tested using the EUROLINE® EBV IgM and IgG blot. One of the 67 samples did not have enough volume for follow-up.
Regarding the interpretation of the EBV serological status, the blot characterised 50 of 66 samples similar to IMMULITE® and 14 of 66 samples similar to LIAISON®.
Concerning all 956 concordant and discordant results these data demonstrated that as well the IMMULITE® EBV VCA IgM and EBNA IgG assays as the LIAISON® EBV results demonstrated respectively 98% and 95% agreement for the interpretation of the EBV serological status.
Conclusions: These data indicate that as well the IMMULITE® EBV VCA IgM and EBNA IgG assays as the LIAISON® EBV results demonstrated good agreement for the interpretation of the EBV serological status.
The IMMULITE® 2000 EBV automated assays would be suitable for EBV testing.

P1920 Performance of the Focus Diagnostics second-generation Parvovirus IgG and IgM ELISA kits utilising parvovirus V9 viral protein 2 antigen

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Objectives: Due to the high degree of homology (98%) between viral protein 2 (VP2) from parvoviruses B19 and V9, assays utilising VP2 from V9 can detect antibodies induced by infection with either B19 or V9 parvoviruses. Focus Diagnostics developed new Parvovirus IgG and IgM ELISA kits that utilise V9 VP2 antigen. We sought to determine the performance of these second generation kits in a reference laboratory setting (the kits are not FDA approved).
Methods: Selected sera (N = 88) submitted for parvovirus serology were evaluated using Biotrin's B19-based Parvovirus IgG and IgM ELISA kits and Focus' new V9-based Parvovirus IgG and IgM ELISA kits. To estimate the adult seroprevalence of parvovirus antibodies using the Focus kits, 41 de-identified sera from healthy volunteers were evaluated. To assess potential cross-reactivity, sera containing antibodies to other infectious agents were tested.

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. Seroprevalence 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.

P1921 Evaluation of the new versant HIV-1 RNA 1.0 Assay (kPCR) for quantification of human immunodeficiency virus type 1 RNA

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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 COBASAmpliprep/COBASTaqMan HIV-1 CAP-TCM (Roche Diagnostics) procedures. Versant HIV-1 RNA 1.0 (kPCR) and COBASAmpliprep/COBASTaqMan HIV-1 assays are both based on RT real-time PCR technology; Versant HIV-1 RNA v3.0 is based on bDNA 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 out-patient care facility of the "Lazzaro Spallanzani" Hospital in Rome. Quantitative results were compared with correlation, linear regression, Bland&Altman 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 0.2 log10 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.

P1922 Significance of QuantiFeron®-TB Gold test for patients infected with HIV-1

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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 are 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-4 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 is that virus changes the tropism for TCD8+and affects all susceptible cells.

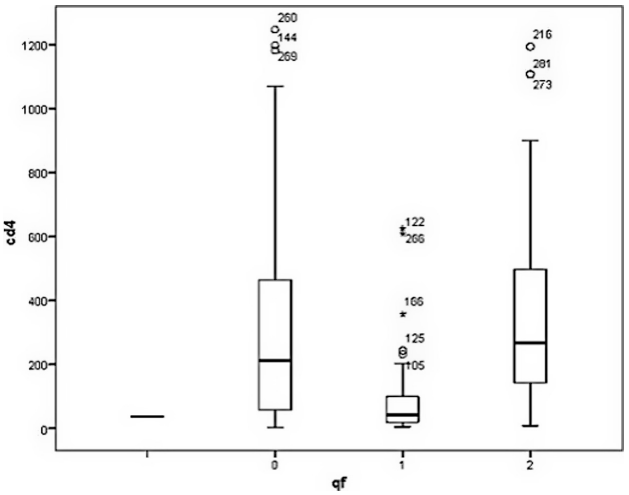

Figure 1. QuantiFeron test–HIV viral load association $p = 0.02$ (QF: 1 = negative; 2 = positive, indeterminate was not considered).

P1923 Performance evaluation of VIDIA® HIV DUO, a new automated immunoassay test for qualitative HIV antigen/antibodies detection

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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 enable 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 reacted 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 0.5 IU/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 routine screening.

P1924 Assessment of the Siemens ADVIA Centaur® HIV Combo assay for routine use in a virology laboratory

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Objective: To compare the performance of the new Siemens ADVIA Centaur HIV Combo assay (CHIV) with the Siemens ADVIA Centaur Enhanced HIV 1/O/2 (EHIV) 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 EHIV assays were run on the ADVIA Centaur XP Automated Immunoassay System. Discrepant samples were centrifuged and duplicate tested with EHIV 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 PRB952. 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 p24 mIU/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.

P1925 Comparability of results obtained with ELECSYS Anti-HCV and Beckman Coulter HCV-ab using routine laboratory samples

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Objectives: 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%.

P1926 Evaluation of fully automated assays for the detection of anti-rubella IgM and IgG antibodies on the Elecsys® immunoassays system

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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.

P1927 Evaluation of fully automated assays for the detection of Cytomegalovirus IgM and IgG antibodies on the Elecsys® immunoassays system

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Objectives: Cytomegalovirus (CMV) infection is the leading cause of congenital viral infection worldwide with an incidence of 0.15–3.0%. Long-term consequences of foetal/neonatal CMV infection can be severe and may include hearing loss, visual impairment and mental retardation. Early detection of primary CMV infection in pregnant patients is therefore important so that the patient can be correctly managed and counselled. The Elecsys® CMV-IgM and CMV-IgG assays are new, rapid and fully automated diagnostic tests designed for routine use on the Roche Elecsys 2010, MODULAR Analytics E170 (Elecsys module), cobas® e 411 and cobas e 601 analyzers.
Methods: Elecsys CMV IgG and CMV IgM are electrochemiluminescence immunoassays based on recombinant antigens. Comparisons of the Elecsys CMV IgM and IgG tests with current methods were performed in clinical laboratories in Italy, France, Israel, Austria and Germany using a range of clinically predefined samples. Frozen serum specimens were used for sensitivity and specificity studies. In addition, fresh serum samples from unselected routine clinical practice (pregnancy testing or blood donors) were tested.
Results: Results of reproducibility testing for the Elecsys assays showed typical values for the coefficient of variation of ~2% for within-assay, and ~3–5% for between-assay repeatability. Both IgM and IgG assays showed similar specificity and sensitivity to comparator methods in samples from primary CMV infection, past infection and samples with confirmed absence of CMV infection. Less reactivity towards samples containing persistent CMV antibodies was found with Elecsys CMV IgM as compared to reference assays. On samples from clinical routine (blood donors and pregnancy testing; n = 1,668) Elecsys CMV IgG showed excellent correlation with the comparator methods (96.8–99.4%). The Elecsys CMV IgM assay revealed higher specificity (97.0–98.8%) than the reference assays (92.9–96.6%) when testing routine samples (n = 1,646).
Conclusion: The Elecsys CMV-IgM and IgG assays showed good concordance with other test methods In addition, the Elecsys CMV IgM assay revealed higher specificity compared to the comparison assays. The performance evaluation data demonstrated that the high throughput Elecsys CMV IgG and CMV IgM assays are reliable tools in routine diagnosis of Cytomegalovirus infection.

P1928 A ten months' results of a commercial enzyme immunoassay for detection of norovirus in outbreak specimens

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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 1064 diarrhoea specimens were collected for viral and bacterial tests in ten months from patients in nursing homes, hospitals, 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 noroviruspositive. Concerning age groups of patients, the highest noropositivity was detected in specimens from children around of 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 during 3 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 norovirusgroups, could be recommended (as screening test) for noro diagnosis in outbreak samples, but not for single-specimen in microbiological laboratories.

P1929 Automated Enzygnost Rubella portfolio (Siemens Healthcare Diagnostics) as an important aid during 2008 rubella outbreak in Italy

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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 Enzygnost Rubella IgG and Enzygnost 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 suspective and they were retested by Vidas Rub 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 BEPIII 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.

ELISA	ELFA		
	Negative	Positive	Border-line
Positive (127 sera)	9 (7.1%)	111 (87.4%)	7 (5.5%)
Border-line (61 sera)	22 (36.0%)	9 (14.8%)	30 (49.2%)
Suspective (112 sera)	112 (100.0%)	0 (0.0%)	0 (0.0%)

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

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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 Borna 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 Bakirkoy Mental Health Hospital.100 sera from healty 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 ug/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). Borna virus 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 Bakirköy 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 psychatric 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

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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

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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.

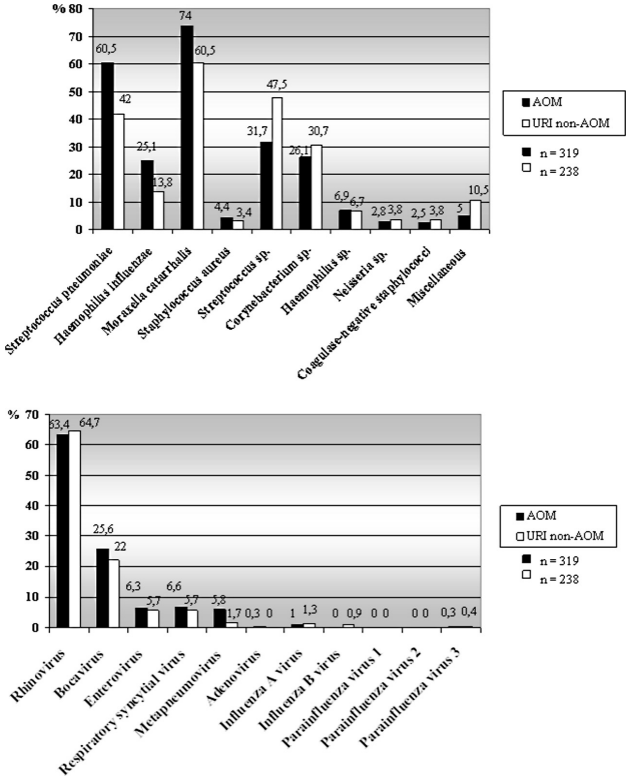

Figure 1. Bacterial and viral findings in the nasopharynx of patients with acute otitis media (AOM) vs patients with upper respiratory infection without AOM.

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.

P1933 Serotype distribution, antibiotic resistance of strains causing acute otitis media and antibiotic comsuption in children in Slovakia

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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.

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 verity of 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 88.5%. 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, 3 strains serotype B and 1 strain serotype F. In total 2 strains (8%) were resistant to ampicilin.

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.

P1934 Clonality and pilus protein genes of pneumococcal isolates causing acute otitis media in children

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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 cultivated 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=3), and those with only NPA positive for pneumococci (MEF–/NPA+, N = 34). 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 (pitA-sipA) 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

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

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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, L'Etoile, 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.

Patients' data

Number of patients	
Male	31
Female	23
Age	1 month to 20 years (mean 52.6 months)
Diagnosis	
Pneumonia	23
Pleuropneumonia	31
Other chronic disease	
Somatomental retardation	6
Congenital vitium cordis	5
Prematurity	4
Gastro-oesophageal reflux	4
Diabetes mellitus	2
Status post renal transplantation	2
Asthma bronchiale	2
Morbus Down	1
Admission	
From home	19
From other hospital	30
From other ward of Department of Paediatrics	4
Antibiotic use before admission	46 (85%)
Length of stay in ICU	2–48 days (mean 7.4 days)
Antibiotic therapy	
Cefalosporins	23
Penicillins	11
Macrolides	10
Aminoglycosides	5
Trimethoprim/sulfamethoxazole (suspected *Pneumocystis carinii* infection)	2
Glycopeptide	1
Carbapenem	1
Lethality rate	5.5%
Aetiology	
Streptococcus pneumoniae	8
Haemophilus influenzae	4
Pseudomonas aeruginosa	4
Staphylococcus aureus	2
Klebsiella pneumoniae ESBL+	1
Chlamydia pneumoniae	1
Candida krusei	1
Anaerobes	2
RSV	3
Pathogen not identified	28

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

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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 for 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 ($P < 10^{-3}$). 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 Pneumovir 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. Papavasileiou, I. Varzakakos, S. Chatzipanagiotou, 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 IgG, IgM – VIRCELL) in order to detect simultaneously specific (IgM, IgG) antibodies against 4 atypical bacteria (*L. pneumophila*, *M. pneumoniae*, *C. burnettii*, *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 38children (43%). Mixed infections were found in 50 children (57%): RSV + Adenovirus (20), RSV + Adenovirus + *M. pneumoniae* (15), *M. pneumoniae* + Adenovirus + Infuenza B (5), RSV + Influenza A

(5) and *M. pneumoniae* + RSV (5). RSV was the most common virus (33%) followed by Adenovirus (30%), Influenza A (9%) and Influenza B (9%). *M. pneumoniae* was the most frequent atypical microorganism detected (15%), followed by *L. pneumophila* (3%), *C. burnettii* (1%) whereas *C. pneumoniae* was not detected. Only 24 children (27.3%) had antibodies of acute-phase. RSV was more frequent in children aged 3 months to 1 year, while *M. pneumoniae* was detected in older patients (4–6 years). Infection was more frequent in winter months, especially in January and February.
Conclusions: RSV was the prevalent causative agent of bronchiolitis in children <1 year, frequently associated with Adenovirus. *M. pneumoniae* was very common in atypical pneumonia and usually associated with Adenovirus or Influenza A and B. Due to the high prevalence of co-infections, the empiric administration of antimicrobial (such as macrolides) to children with LTRIs, might contribute to the treatment and the prevention of relapses.

P1938 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. Iliadou, A. Voyatzi (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 IVC's. 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 (BioMerieux, 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 stain showed intermediate susceptibility to teicoplanin. Multiresistance was detected in *Acinetobacter baumannii* 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.

P1939 Epidemiology of *Moraxella catarrhalis* infections in children and resistance phenotypes

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Objectives: To investigate the incidence and the antibiotic resistance phenotype patterns of *Moraxella catarrhalis* strains isolated from respiratory tract infections and to evaluate the burden of disease in childhood.
Material and Methods: A total of 200 *M. catarrhalis* strains recovered from children aged 0 to 14 years during a four year period (2005–2008). Identification of *M. catarrhalis* strains was based on the production of DNAse and the tributyrin hydrolysis reaction. Antimicrobial susceptibility performed through the disk diffusion method and confirmed by E-test (AB Biodisk). β-lactamase production determined by a chromogenic cephalosporin test.
Results: *M. catarrhalis* strains isolated in 32% clinically diagnosed respiratory tract infections (RTIs). The origin of these 200 strains was: sputum 38%, bronchoalveolar lavage (BAL) 30%, ear smears 15%, nasal secretions 11% and conjunctival smears 6%. It is notable that a rise of incidence of otitis media and bronchiolitis, followed by conjunctivitis, observed during autumn and winter concerning children ages <3 years. All *M. catarrhalis* strains were sensitive to: amoxicillin/CA (AMC) (MIC50 0.064 mg/l-MIC90 0.50 mg/l), ciprofloxacin (MIC50 0.047 mg/l-MIC90 0.125 mg/l), cefuroxime (MIC50 0.25 mg/l-MIC90 1 mg/l), and imipenem (MIC50 0.064 mg/l-MIC90 0.19 mg/l). A low rate of resistance to chloramphenicol 3% (MIC50 0.25 mg/l-MIC90 1 mg/l), tetracycline 6% (MIC50 0.25 mg/l-MIC90 1 mg/l) and trimethoprim/sulfamethoxazole 8% (MIC50 0.125 mg/l-MIC90 0.50 mg/l) determined. Remarkable resistance exhibited to erythromycin (23.3%, MIC50 0.094 mg/l-MIC90 1.5 mg/l) while resistance to newer generation macrolides was: to clarithromycin 30.9% (MIC50 0.094 mg/l-MIC90 3 mg/l) and to azithromycin 16.6% (MIC50 0.023 mg/l–MIC90 1 mg/l). All *M. catarrhalis* strains resistant to ampicillin (90%), were producing β-lactamase.
Conclusions: 1.A seasonal distribution of *M. catarrhalis* infections incidence observed, according to sample origin. 2. In children with severe respiratory consequences consecutive quantitative sputum cultures obtained in order to clarify whether it was a simple colonisation or an active infection. 3. *M. catarrhalis* strains were recovered from 30% of children with chronic obstructive pulmonary disease as the predominant pathogen. 4. β-lactamase producing strains presented a considerable MIC increase to clarithromycin. 5. AMC remains the appropriate antibiotic of choice due to the appearance of resistance to macrolides.

P1940 Prevalence and antimicrobial susceptibility among *Pseudomonas aeruginosa* strains isolated in a paediatric hospital

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Objectives: to evaluate the prevalence and to investigate the antimicrobial resistance patterns of *Pseudomonas aeruginosa* (P.a.) strains isolated from hospitalised children with severe infection during a four year period (2005–2008).
Material and Methods: Totally, 472 P.a. 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-Mèrieux). Metalo-β-lactamases (MBL) production was screened by E-test MBL-strip (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 P.a. strains to 12 antibiotics ranged: ticarcillin (TIC) 33%, netilmicin (NET)18.9%, aztreonam(AZT) 14.1%, piperacillin (PIP) 14%, ceftazidime (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) 6.5% and piperacillin-tazobactam (TZP) 4.7%. Most of P.a. 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 P.a. strains from ICU samples while resistance rate to carbapenems was 11.1% (MIC50

2 mg/l-MIC90 8 mg/l) because a percentage of MBL producing strains (7.8%) were found. Among the resistant to aminoglycosides P.a. strains, AAC (6′-1) and ANT (2”-1) enzymes were predominant.
Conclusions: 1. The majority of P.a. strains was associated with surgical wounds (60%) followed by low respiratory tract infections (25%). 2. The overall proportion of P.a. isolates from ICU patients presented multiple resistant phenotypes including even resistance to carbapenems. 3. Different mechanisms of resistance are involved in the expression of multiple antibiotic resistant phenotypes, especially in ICU patients. 4. Monitoring of antibiotic sensitivity among ICU P.a. strains may prevent the spread of resistance in paediatric clinics.

P1941 Anaerobic bacteriology and antibiotic susceptibility of intra-abdominal community-acquired infections in children

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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 intraperitoneal and surgical wound infections over a two years 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 5.5% and mixed aerobes and anaerobes 30%. The anaerobes bacteria isolated were: *Bacteroides fragilis* group (Bacteroidales-32%), Peptostreptococci (28%), *Clostridium* spp (14%), *Prevotella* spp. (8%), *Bacteroides capilosus* (6%), *Fusobacterium* spp (5%), *Vellionella* spp (4%) and *Porphyromonas* spp (3%). *Bacteroides 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, *Bacteroides 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), piperacillin-tazobactam (14.2%, high MIC >256 mg/l) and cefoxitin (7.1%, MIC 96 mg/l), while a total of 17.8% strains showed intermediate susceptibility to cefoxitin (MIC 32 mg/l) and 3.5% to metronidazole (MIC 16 mg/l) and tigecycline (2%).
Conclusions: 1. Polymicrobial mixed aerobes and anaerobes were responsible for half of established peritonitis cases so the antibiotic therapy should provide coverage for both types of pathogens. 2. *B. fragilis* is the most frequently isolated anaerobic pathogen in IAIs. 3. Imipenem is the most potent agent tested while the emergence of resistance to metronidazole has important implications in the treatment of cIAIs and PSWIs. 4. Multiple resistance phenotypes of anaerobes emerge the issue of surveillance necessity for antimicrobial susceptibility.

P1942 Viral, bacterial and parasitic aetiology of acute gastroenteritis in hospitalised children in north-western Greece

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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 pathogens 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 Drove, 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, *Yersinia enterocolitica* in 4, *E. coli* in 2, *Aeromonas hydrophila* in 2), while single viral infections were identified in 1338 children (74.8%). Viral-bacterial coinfection was found in 26 (1.4%) cases and viral-viral coinfection in 36 (2%) cases. No sample was positive for parasites. Viral pathogens were identified in 1400 children (30.4%): Group A rotavirus was detected in 983 (21.35%) cases (941 monoinfections, 32 virus-virus coinfections and 10 virus-bacteria coinfections), adenoviruses in 162 (3.5%) cases (142 monoinfections, 16 virus-virus coinfections and 4 virus-bacteria coinfections), 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.

P1943 Infectious complications in paediatric acute liver failure, a single-centre experience

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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 (Pfts;< 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.

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

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Objectives: Gastro-oesophageal reflux disease (GERD) is defined as symptoms or complications of reflux of gastric content into the oesophageus. 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 (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-hours pH-monitoring and the Infant Gastroesophageal 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

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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 cystouretrography (VCUG)during an 11 year period. Altogether 8567 patients were identified and 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 data 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 424/2036 (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

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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 symptomatic. Vesicoureteral 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, CI1.42–12.37, $p = 0.01$) 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 stabile 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

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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%), *Enterococcus*

spp (4.2%) and *P. aeruginosa* (3.0%). The most frequent species of the remaining 7.2% of isolates were *E. cloacae*, *C. albicans*, *K. oxytoca*, *A. baumannii*, *Citrobacter* spp, Coagulase Negative Staphylococci (CNS) and *S. aureus*. The incidence of *E. coli* in non hospitalised children reached 77.8%, followed by *P. mirabilis* (13.4%) and *K. pneumoniae* (4.7%). In hospitalised children, the most frequent isolate was *E. coli* (66.1%), followed by *K. pneumoniae* (7.8%), *Enterococcus* spp (5.6%), *P. mirabilis* (5.2%) and *P. aeruginosa* (4.3%). *P. mirabilis* was more frequent in girls (54.1%) than in boys. 68/72 *P. aeruginosa* and 16/17 *C. albicans* strains were isolated from hospitalised patients. The vast majority of these strains were isolated from children with vesicoureteral reflux (3rd grade or more), surgery in the urinary tract, immunosuppression, prematurity or prolonged hospitalisation.

Conclusions: *E. coli* was the predominant causative pathogen of urinary tract infections in children, with an increased prevalence in non hospitalised patients. The higher isolation rate of *P. mirabilis* in girls in this study (54.1%) shows that boys are not the great preference of this uropathogen. This finding indicates that positive urine cultures with *P. mirabilis* in boys must be evaluated with particular prudence. There is a low frequency of mixed urinary tract infections in children.

P1948 Outcome of acute childhood bacterial meningitis in Luanda, Angola

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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 $\geqslant$80 dB). Hearing was measured using brain stem auditory evoked response.

Results: The preset 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 observed in 25%, extrameningeal focus of infection in 26%, dyspnoea in 51%, malnourishment in 28%, positive malaria test in 29% and blood haemoglobin <8 g/dL in 55%. Of those tested, 7% were HIV-positive. Of the 428 analyzed cases, 177 (41%) were caused by *Haemophilus influenzae* type b (Hib), 171 (40%) by *Streptococcus pneumoniae* (Pnc), 49 (11%) by *Neisseria meningitidis* (Mnc) and 31 (7%) by other (mostly Gram-negative) bacteria. The overall case fatality was 37%; 32% in Hib, 45% in Pnc, and 16% in Mnc meningitis. Of survivors 13% were left with SeNeSe; 13%, 17% and 2% in Hib, Pnc and Mnc meningitis, respectively. Deafness at discharge was found in 18% (47/267); 21%, 21% and 9% in Hib, Pnc and Mnc meningitis, respectively. In the group of "other bacteria" only 12 of 31 children (39%) survived, 10 of whom without sequelae. When controlled 1 month later, 13% of children had SeNeSe, 8% were deaf and 2 children had died.

Conclusions: BM of childhood continues to be a major problem in Angola. Fatality was highest in meningitis caused by Pnc or Gram-negative bacteria, and lowest in Mnc meningitis. Importantly, no recovery from SeNeSe occurred during the first month post-hospitalisation, whereas deafness decreased from 18% to 8%. Since such improvement in hearing has not been found previously, we are looking for explanations to this positive finding.

P1949 Invasive meningococcal disease in children

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Neisseria meningitidis is a well-known cause of acute meningitis and fatal sepsis in healthy children worldwide.

Objectives: 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.

Methods: Retrospective review of medical records of all children aged $\leqslant$18 years with IMD hospitalised in our clinic from 1998 through 2007.

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 in 150 patients (79%), rash in 127 (67%), hypotension in 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. Only 77 (40.3%) of all cases was treated before admission, 60 (77.9%) of them receiving preadmission β-lactamins. The treatment was based upon penicillin G (49%). All isolates were sensitive to penicillin. The average duration of antibiotic therapy was 8±4.3 days (m±SD). In 97 cases (51%) we administrated cortisone therapy.

Conclusion: Due to variable clinical manifestations and preadmission antibiotherapy, early diagnosis is sometimes difficult; that's why laboratory confirmation should be improved by the introduction of PCR-based techniques.

P1950 Ampicillin or penicillin for empiric treatment of early-onset neonatal sepsis: influence on bowel colonisation by aerobic and facultative anaerobic bacteria

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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 terms 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 and needing empiric therapy with ampicillin or penicillin and gentamicin. Microbes were identified on the genus and species level using biweekly collected rectal swabs. The ratio of colonising days to 100 PICU days (total colonisation density – TCD) and number of patients colonised was used to compare the two regimens.

Results: Each treatment group included 139 neonates. Compared to penicillin the mean TCD of *Klebsiella pneumoniae* and *Candida* spp. was higher but 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 Eneterobacteraciae in both treatment groups was similar. Multivariate logistic regression analyses 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).

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

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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 tuberculous 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. 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 hypertransaminaemia. 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

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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*

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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 gonorrhoea (*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, chlamydia 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 neonatorom, 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 Giemsa 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 neonates 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

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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 occured 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), MLSBi (inducible resistance) 8.75% (7/80) and MLSBc (constitutive resistance) 2.5% (2/80)]. Of *S. pyogenes* isolates, 23% were resistant to macrolides [MLSBi 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*.

P1955 Prevalence of coronavirus subtypes infection in paediatric patients by real-time RT-PCR in Slovenia

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Objectives: Acute respiratory viruses are a major cause of morbidity and mortality in humans worldwide and most acute respiratory infections are caused by viruses. It is estimated that 10% of all viral respiratory tract infections of hospitalised children are caused by four genotypes of human coronaviruses (HCoVs): HCoV-229E, HCoV-OC43, HCoV-HKU1 and HCoV-NL63. Coronaviruses are enveloped, positive-strand RNA viruses belonging to the order Nidovirales, family Coronaviridae and genera Coronavirus. The aim of the present study was to estimate the prevalence of HCoVs in a cohort of hospitalised infants and young children with symptoms of respiratory tract disease from June 2007 to May 2008.
Methods: The samples (1076) from children (834) were sent to laboratory for routine detection of respiratory viruses. From 1076 samples there were, 717 (66.6%) nasopharyngeal swabs, 228 (21.2%) throat swabs, 92 (8.5%) tracheal aspirates and 39 (3.6%) others. Female to male ratio was 1:1.35. All patients (834) were distributed in to five age groups. Total nucleic acid was isolated from 200 µl of each respiratory specimen on MagNa Pure Compact (Roche Diagnostics, Mannheim, Germany) according to manufacturers instructions. All four human coronaviruses were detected by real-time RT-PCR assay using eight primers and 3 MGB probes to amplify 85- to 100 bp fragments of the polymerase 1b gene.
Results: Human coronavirus RNA was detected in 5.3% of samples. The highest prevalence of infections (8.3%) was found in children between 2 and 3 years of age. Coronaviruses were detected in every month except June, with a maximum number in February, 20 (35.1%) of 57 positive specimens. Of the 57 specimens positive for a coronavirus, 9/1076 (0.8%) were HCoV-229E, 6/1076 (0.6%) were HCoVNL63, 29/1076 (2.7%) were HCoVHKU1 and 13/1076 (1.2%) were HCoVOC43.
Conclusion: All four genotypes of coronaviruses were detected in samples from hospitalised children. These results confirms that coronaviruses are important human pathogens associated with acute respiratory tract infections in Slovenian children.

P1956 Prevalence of human bocavirus in paediatric patients with respiratory infection in Slovenia

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Objectives: Human Bocavirus (HBoV), was discovered in 2005 in children with lower respiratory tract infections (LRTI). Since then, the respiratory infections with HBoV has been reported worldwide with frequencies ranging from 1.5% to 19.3% in respiratory samples of children. Aim of our 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. For DNA extraction 195 µl of respiratory specimen and 5 µl of Equine herpesvirus 1 (EHV1) as internal control was used. DNA was eluated in 100 µl eluation buffer. DNA isolation was performed on MagNa Pure Compact automatic nucleic acid extraction platform. NS1 region (88 bp) of HBoV DNA was amplified by real-time PCR using Taqman probe. The amount of virus in each sample was assesed quantitatively using a part of cloned HBoV genome as a standard.
Results: Overall, 175 of 820 samples (21.5%) were positive for HBoV by real-time PCR. The highest percentage of positivity was found in children in the age group between 13–24 months (33.3%). A seasonal distribution was evident and number of positive samples rose from October to March with the peak in December.
Conclusion: HBoV was frequently detected in respiratory samples from Slovenian hospitalised paediatric patients with respiratory infection. Although we found the highest percentage (21.5%) of HBoV DNA in paediatric samples so far, 47.4% of those are in the group with the lowest amount of HBoV DNA detected. The study showed that infections with HBoV are more frequent during the late autumn and winter time in children age group between 13–24 month. We confirm that HBoV is widespread respiratory tract infection in children. The clinical significance of the low level HBoV DNA in majority of respiratory samples warrants further studies.

P1957 Clinical course and sequelae in tick-borne encephalitis in children in southern Moravia (Czech Republic)

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Objectives: The aim of the study was to characterise the clinical course of the illness in children and laboratory findings including CSF, to identify neurological sequlae and duration of IgM positivity.
Patients and Methods: We evaluated demographic and epidemiologic data of 94 children, signs and symptoms at admission, clinical course during hospital stay, laboratory findings and sequelae. Serology for neurotropic viruses including TBEV and for antiborrelial antibodies was performed looking for coinfections. CSF was analysed for cells, protein, glucose and TBEV specific IgM antibodies. Children were followed up for at least 12 months.
The data were analysed with StatSoft 7. Quantitative data were compared by Mann-Whitney test and qualitative data by the Fisher's exact test. Two-tailed p-values of 0.05 were interpreted as statistically significant.
Results: A total of 445 children were admitted for aseptic meningitis. 94 cases with TBE represent 21% of the viral neuroinfections among children in the Southern Moravian region.
The clinical course of the illness was biphasic in 61% of cases. 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 conciousness ($p < 0.001$), seizures ($p = 0.03$) and paretic 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 no known relationship between the sequlae 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

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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 transplacenta 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 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)

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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 case numbers 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.

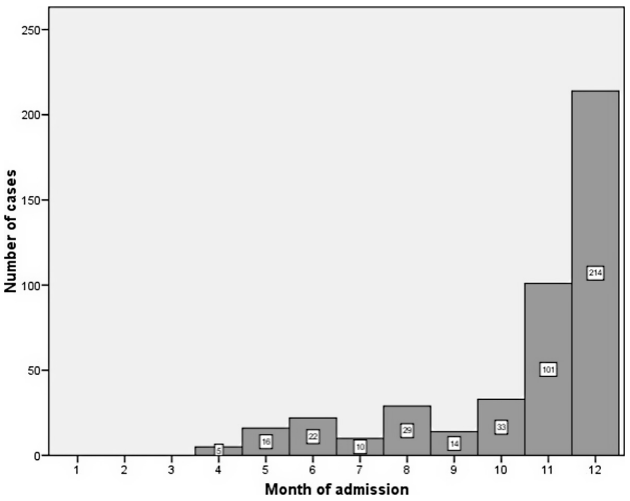

Figure 1. Measles occurrence by month of admission.

Conclusions:
1. Despite the isolation measures, 15% of our patients developed nosocomial measles, probably due to the very high contagiosity 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%.

P1960 **Aetiology of fever in children from urban and rural Tanzania**

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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 >38ºC 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 by the rapid test for most of them. Malaria confirmed to be lower than generally thought. Results of molecular analyses and serologies will be presented and will provide further insight on the respective contribution of bacteria and viruses, a critical issue for appropriate management of fever and rational use of antibiotics.

Fungal epidemiology

P1961 **Candidaemia in Finland, 1995–1999 versus 2004–2007**

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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. during 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.
Conclusions: 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.

P1962 **The pattern of candidaemia in a tertiary referral hospital in the United Kingdom**

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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. 88% of episodes were hospital acquired and 51% of episodes were from intensive care units (ICU). Non-*Candida albicans* species comprised 57% 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.

P1963 **Prevalence of *Candida metapsilosis* and *Candida orthopsilosis* isolates in a Spanish yeast stock collection**

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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. metapsilosis* 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 *C. parapsilosis* 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.
Funding: Projects GIC07 123-IT-222–07 (from the Departamento de Educación, Universidades e Investigación, Gobierno Vasco) and PI061895/2006 (from the Fondo de Investigación Sanitaria del Ministerio de Sanidad y Consumo de España).

P1964 Do candiduria predict candidaemia in intensive care units in a Danish tertiary-care hospital?

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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 (8%) 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

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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* aortic 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, flucytosine, 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 precribed prolonged fluconazole at discharge post surgery.

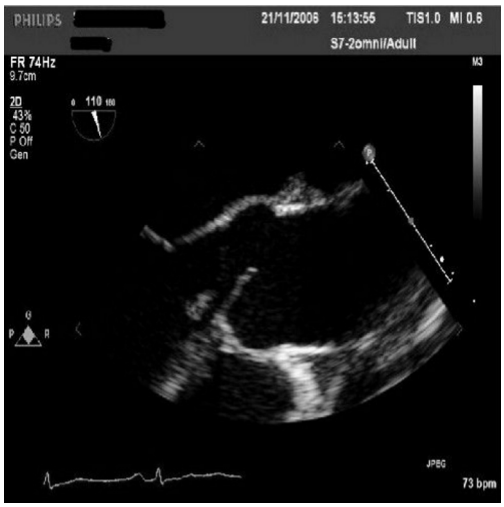

Figure 1. TOE 21/11/06 confirming vegetation affecting 2 leaflets of the aortic valve associated with severe aortic regurgitation. Some oedema of the aortic valve annulus was noted with suspicion of early abscess formation. 1 cm vegetation noted.

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* endocarditisis. 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.

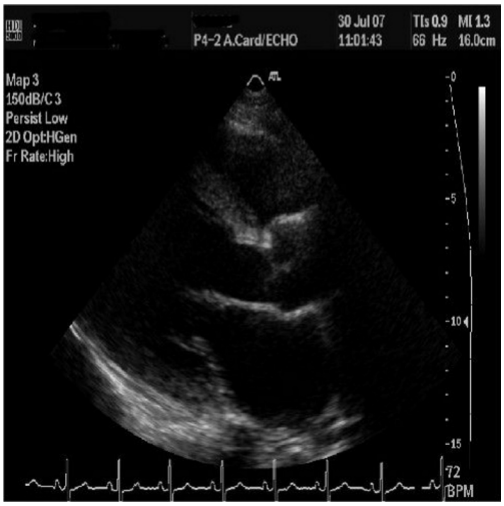

Figure 2. 30/7/07 Vegetation hardly noticeable, possible prolapse of left coronary cusp. Severe regurgitation.

P1966 Cryptococcal meningitis: 12-year experience in a single tertiary-care centre

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Objectives: Cryptococcal infections are frequent in HIV-infected patients and are regulary 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 cerebral spinal 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 mmH20) 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.

P1967 Cryptococcal infections in non-HIV-infected patients: description of 9 cases collected in a tertiary hospital, 1989–2008

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Background: Cryptococcosis in the last 25 years has mainly affected HIV-infected patients. Other debilitating conditions (e.g. haematological cancer, solid organ transplantation, rheumatic disease) were much less common among patients with cryptococcosis. We describe nine non-HIV-infected patients with cryptococcosis, with emphasis on the clinical manifestations, laboratory and radiological findings, microbiological diagnosis, and outcome.
Methods: We reviewed the charts of nine patients with cryptococcosis (isolation of *Cryptococcus* from ordinarily sterile sites or respiratory samples) and non-HIV predisposing conditions (1989–2008).
Results: We collected data on 58 patients with CCS during the study period, and 9 (15.5%) patients had no-HIV infection. A total of five patients were male and their age ranged from 21 to 80 years. The predisposing conditions were solid organ transplant (3, 33.3% [2 renal, 1 heart]), chronic lymphocytic leukaemia (1, 11.1%), cirrhosis by HBV (1, 11.1%), biliary-tract carcinoma (1, 11.1%), and rheumatologic disease (2, 22.2% [1 rheumatoid arthritis and 1 alveolar proteinosis]) and none (1, 11.1%). We only detected one case of infection in an apparently immunocompetent subject caused by *C. gattii*. Six of the patients were receiving corticosteroid therapy and five were receiving immunosuppressive agents. The clinical forms were neurocryptococcosis and meningitis (4, 44.4%), fungaemia + meningitis (1, 11.1%), fungaemia + pulmonary disease (2, 22.2%), and fungaemia alone (2, 22.2%). Fever (n = 8) and headache (n = 4) were the most frequent symptoms. The analytical CSF parameters were (in ranges) opening pressure (18–30 cm H2O), leukocytes (0–313 cells/mm^3), glucose level (9–51 mg/dl), and protein level (25–319 mg/dl). Cryptococcal antigen was detected in 6 patients (all patients with meningitis and two with fungaemia). The distribution of aetiological agents was: *C. neoformans* var. grubii (n = 6, 66.6%), *C. neoformans* var. neoformans, (n = 2, 22.2%), and *C. gattii* (n = 1, 11.1%). All patients responded to antifungal treatment (amphotericin B, flucytosine, or oral fluconazole).
Conclusions: Cryptococcosis in non-HIV-infected patients is a very uncommon fungal infection, occurring mostly in patients receiving corticosteroids. Patients with cryptococcosis and non-HIV infections had meningitis in only half of the cases and presented a good outcome. *C. neoformans* var. grubii was the most common causative agent.

P1968 Non-bacillary *Mycobacterium tuberculosis* pneumonia preceding *Cryptococcus neoformans* co-infection in an otherwise healthy young girl

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Introduction: The concurrence of cerebral tuberculosis and cryptococcosis is an exceedingly rare event without a severe underlying immunosuppression.
Case report: A 25-year-old student recently immigrated from China was admitted due to a presumed meningoencephalitis. Epidemiological features and clinical history were mute. Since cerebrospinal (CSF) fluid examination showed pleocytosis with lymphocyte predominance, increased albumin content, and low glucose, and all microscopic, culture, and antigen search for *Cryptococcus neoformans* tested positive, an antifungal treatment was started with high dose fluconazole. HIV serology tested negative, but a moderate impairment of CD4+ lymphocyte count was detected (299 cells/μL). Due to a persisting clinical-neurological-CSF impairment, liposomal amphotericin B replaced fluconazole. The appearance of cranial nerve deficits paralleled the culture isolation of *Mycobacterium tuberculosis* from the first CSF specimen, while intradermal tuberculin testing remained negative, and cryptococcosis was microbiologically cured. Serial contrast-enhanced brain CT scans showed the appearance and the worsening of multiple flogystic frontal, hypothalamic, and ponto-cerebellar leptomeningeal lesions, confirmed my MRI scans. A 5-drug anti-tubercular therapy was immediately

started and subsequently potentiated with quinolones and linezolid. After 5 months of hospitalisation, thanks to an extensive rehabilitation program, a slow ameliorement 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 underestimation 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

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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

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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 jirovecii* 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 pathogens 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 paranasal 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

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Objectives: Identify risk factors associated with zygomicosis 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: haematological 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 ($<500\,mm^3$). 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.

P1972 Filamentous fungi in cystic fibrosis: occult *Scedosporium* colonisation detected by selective media

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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 56 patients (median age 24 years, IQ range 21–28years, 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. flavus* (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. flavus* 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.

P1973 Fungi from airway secretions of children with cystic fibrosis

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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 from 393 children with CF (mean age 8.9±3.8 years) were performed. Sabouraud dextrose agar (Conda Pronandisa, Spain), with Gentamicin, was used for the culture of fungi, incubated at 35ºC for up to 5 days. The identification of filamentous fungi was based on the macroscopic and microscopic morphological features in accordance with standard descriptions. Identification of yeasts was based on the carbohydrate's assimilation (API 20 AUX, Biomerieux).
Results: 426 cultures from 221 children were positive for fungi (15.4%). Multiply isolates of the same fungus per patient were included only once. The prevalence of potentially pathogenic fungi from patients with CF as *Candida albicans*, *Aspergillus fumigatus*, *Scedosporium apiospermum* and *Exophialla* (*Wangiella*) *dermatitidis* was 62.9, 11.8, 3.8, and 0.4% respectively. All species of fungi isolated from the 221 children are presented in the 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 *Exophialla* (*Wangiella*) *dermatitidis*.

Fungi from airway secretions of patients with CF

Species	Number	%
C. albicans	149	62.9
C. parapsilosis	17	7.2
C. lusitaniae	3	1.3
C. famata	3	1.3
C. kefyr	1	0.4
A. fumigatus	28	11.8
A. terreus	12	5.1
A. flavus	9	3.8
A. versicolor	1	0.4
A. oryzae	1	0.4
Scedosporium apiospermum	9	3.8
Exophialla (*Wangiella*) *dermatitidis*	1	0.4
Geotruchon spp.	1	0.4
Cryptococcus laurentis	2	0.8
Total	237	

P1974 *Scedosporium apiospermum* from airway secretions of children with cystic fibrosis

H. Alexandrou-Athanasoulis, S. Doudounakis, A. Stathi, A. Katelari, A. Pangalis (Athens, GR)*

Objective: *Scedosporium apiospermum* has recently been included to the fungal agents which may be of clinical importance in patients with cystic fibrosis. Aim of the study: The incidence of airway colonisation/infection by Scedosporium apio-spermum in comparison with clinical data and the MICs of 5 antifungal agents (itraconazole, ketoconazole, voriconazole, posaconazole and gaspofungin) against the isolated species.
Material and Method: Over a twenty three months period (February 2007 – C December 2008) 2770 sputum or deep throat cultures were performed to 393 children (mean age 8.5±3.9 ys) with cystic fibrosis attending our department. For the detection of fungi the Sabouraud dextrose agar (Conda Pronandisa, Spain) with gentamicin was used, incubated at 35ºC and examined daily for fungal growth for up to 5 days. The identification of *Scedosporium apiospermum* was based on the macroscopic and microscopic morphological features in accordance with standard descriptions. The MICs of antifungal agents were determined with the gradient MIC method using E-test strips (75% reduction of growth). *C. parapsilosis* ATCC 22019 was used as quality control.
Results: Positive for *Scedosporium apiospermum* were 57 cultures (2.1%) from 9 children (2.3%) (mean age 12.5,±0.5 ys). Five children are constantly colonised and four seem to have a transient colonisation. Seven of them have a very good clinical status (with FEV1 and spirometric findings within normal values) while one has allergic bronchopulmonary disease. The cultures of the last patient have yielded *A. fumigatus*, *Exophialla dermatitidis*, *C. albicans A. terreus* and *Cryptococcus* laurentis as well. The MICs (mg/L) of 5 antifungal agents were: itraconazole: 0.008–0.5, ketoconazole: 0.0012–0.9, voriconazole: 0.006–0.047, posaconazole: 0.008–⩾32 and caspofungin: 0.003–⩾32).
Conclusions: 1. The airways surveillance of CF patients should include suitable culture methods for the isolation of *Scedosporium apiospermum* from sputum cultures. 2. Although the numbers of isolates tested to antifungal agents are low, it seems that voriconazole is the most active antifungal agent against *Scedosporium apiospermum*.

P1975 Clinico-mycological profile of onychomycoses in patients attending dermatology clinics at a tertiary care centre in southern India

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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 Onychocola canadensis, Lasiodiploidia 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.

P1976 Onychomycosis in Tehran: mycological study of 504 patients

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Background: Onychomycosis, a common nail disorder results from invasion of the nail plate by a dermatophytes, yeasts or mould species that these fungi give rise to some diverse clinical presentations.
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 dublicate for dermatophyte and (3) SDA with 5% chloramphenicol triplicate for mould isolation.
Results: Out of a total of 504 cases examined, 216 (42.8%), were mycologically proven cases of onychomycosis (144 finger nails, 72 toe nails). Among the positive results, dermatophytes were diagnosed in 46 (21.3%), yeasts in 129 (59.7%) and non dermatophytic mould in 41(19%). *Trichophyton mentagrophytes* was the most common causative agent (n = 22), followed by *Trichophyton rubrum* (n = 13), *Candida albicans* (n = 42), C. spp. (n = 56) and *Aspergillus* spp. (n = 21).
Conclusions: near the half of clinical suspected fungal nail infections is onychomycosis and yeast is responsible for most of the infections in Iran.

Pharmacokinetics & pharmacodynamics of aminoglycosides & lipoglycopeptides

P1977 In vitro pharmacodynamic properties of colistin methanesulfonate and amikacin against *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis

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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 using time-kill curve method. PAEs were determined by the standard viable counting method where bacteria in the logarithmic phase of growth were exposed for 1 hour to the antibiotics at 1× or 20×MIC, alone and in combinations.
Results: Synergy and additive effects were detected with colistin methanesulfonate and amikacin combination, at 1×MIC concentrations, at 24 h. Some of the strains produced an earlier synergistic effect at 12 h. No antagonism was observed with any combination. Colistin methanesulfonate at 1× and 20×MIC concentrations produced PAEs 1 to 2,85 h, amikacin at the same concentrations produced 0.6 to 3.75 h. 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 4.8 h.
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

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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 absortion (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±3.6 h and Clearence (CL):8.8±6.4 L/h for patients with TBSA < 20%, and VD: 0.5±0.6 L/kg, t1/2:4.40±3.70 h and CL:8.50±13.90 L/h for patients with TBSA ⩾ 20%. After the pharmacokinetic adjustment, amikacin doses had rised to 34.0±20.0 mg/kg/day.

Conclusions: As expected, these results show a great interindividual variation in amikacin pharmacokinetic parameters. Taking the trough/peak values into account, the standart 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

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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 nephrotoxic 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

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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 appropriate or inappropriate, 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 Cockroft-Gualt 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 total 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

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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 (1–8kH) on at least 3 occasions: at the start, during the course and after the cessation of G 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 20dB 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

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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 antimicrobial 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 target attainment (TA) of 15–20 mg/L, 66.4% achieved TA of 14–21 mg/L, while 73.4% patients achieved TA of 13–22 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

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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 were collected the following variables: demographics, infectious disease, clinical situation at the beginning of treatment (mechanical ventilation, vasoactive drugs and renal function) and through serum concentration (corresponding to sample just before the next dose) estimated in stationary state (Cpss).
The Cpss target was defined 4–8 × MIC; in pneumonia AUC/MIC > 350; and in absence of antibiogram the target was 10–20 mg/L. Vancomycin serum concentration was determined by Axym® (Abbott®) autoanalyzer. Pharmacokinetics analysis was performed through the package PKS® (Abbott®). The results are expressed as mean (standard deviation).
Results: 34 patients were included (M:W 27:7). The mean age was 51.3 (17.0) years, with a mean weight of 82.8 (25.5) kg and a mean height of 171 (9) cm. The indication of treatment with vancomycin was 35.5% bacteraemia, 18% CNS, 15% pneumonia, intraabdominal infection 6%, 26% others. The mean Cockroft-Wault clearance creatinin was 153 (92) mL/min.
73.5% of patients (n = 25) treated with vancomycin required adjustment of the initial dosing. Patient distribution: 19 had Cpss below target concentration (mean dose: 12.2 (2.7) mg/kg; mean Cpss: 5.0 (2.7) mg/L) and 6 patients had Cpss above target concentration (mean dose: 12.8 (1.7) mg/kg; mean Cpss: 25.3 (4.5) mg/L). 26.5% of patients (n = 9) did not required dose adjustment (mean dose: 13.7 (1.2) mg/kg; mean Cpss: 9.2 (3.1) mg/L).
Conclusion: The high percentage of patients who need a change in dosing suggests a protocol amending the initial dose of vancomycin. Given the wide variability pharmacokinetics observed in these patients and the severity of infections in critical care units, is necessary an extensive monitoring and early pharmacokinetics in order to achieve plasma concentrations effective as soon as possible.

P1984 High-dose daptomycin for complicated Gram-positive infections

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Objective: We evaluated the safety and efficacy of high-dose daptomycin (HD DAP) therapy, defined as ⩾8 mg/kg/day.
Methods: A retrospective case series from 4 academic medical centres. Consecutive patients (pts) treated with HD DAP for ⩾72 h inpt or continued as outpt therapy, excluding dialysis pts, were collected from 2005–2008. Charts were reviewed for demographics, comorbidities, antimicrobial therapy, microbiologic cultures, clinical outcomes, and adverse events.
Results: 105 eligible pts were identified. Baseline characteristics: Median age 54 yrs (range 19–89), 79.0% prior hospitalisation ⩽1 yr, 32.4% renal disease, 30.5% diabetes, 27.6% IDU, 23.8% prior MRSA infection ⩽1 yr. Primary infections: 26.7% complicated bacteraemia, 25.7% bone/joint, 23.8% endocarditis (by Duke criteria), 22.9% skin/wound. 68.6% received HD DAP following non-response to vancomycin (VAN) therapy. Median dose of DAP was 8.2 mg/kg/day (range 8.0–12.1). Median duration of total antibiotic therapy was 17.0 days (range 1–98) and duration of HD DAP was 9.0 days (range 1–49). Median length of hospital stay (LOS) was 18.0 days (range 3–111), duration of bacteraemia 4.0 days (range 1–36). 81.9% had a favourable clinical outcome, defined as clinically improved or cured, and 71.4% (of 96 patients) microbiological eradication. Safety: 80.0% of pts had maximum creatine phosphokinase (CPK) levels ⩽150 IU/L (range 8–1758), and 90.6% had end-of-therapy CPK levels ⩽150 IU/L (range 11–412). Of note, 16.2% pts were on concomitant HMG-CoA reductase inhibitors. No pts required discontinuation of HD DAP because of myopathy. Non-susceptibility to DAP (NSD) developed in 4 pts with MRSA as primary organism, IDU, and non-response to VAN in common. Median total LOS in NSD was 21 days (range 8–30). 3 out of 4 pts were initially treated with VAN for organisms with VAN MIC 2 mg/L for at least 2 days prior to HD DAP therapy. Primary infections for these pts: 2 bone/joint + skin/wound, 1 right-sided endocarditis, and 1 right- and left-sided endocarditis. 3 pts improved on alternative therapy while 1 pt was considered clinical cure + eradication despite DAP MIC 2 mg/L.

Table 1. Initial susceptibility of pathogens

Organism	VAN (mg/L)		DAP (mg/L)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Enterococci (N = 36)	⩾32	⩾64	2	2
MRSA (N = 54)	2	2	0.75	1

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

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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 192 h. Selected VAN dosing regimens simulating fAUC/MIC ratios from 7 to 224 (31–1000 mg q 12 h) were evaluated in duplicate in a one-compartment PK/PD model and 112 (500 mg q 12 h) 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× 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 144 h for the agr II null strain versus 192 h for the isogenic agr+ strain. Mutants of the agr null strain recovered from plates containing 3× the baseline VAN MIC at 192 h 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

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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 hr concentration 3.3 mg/L, 24 hr concentration 1.1 mg/L; teico Cmax 4.5 mg/L, 12 hr concentration 2 mg/L, 24 hr 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 10^6 CFU/ml. Antibacterial effect was measured by log change in viable count at 12 h (d12), 24 h (d24), 36 h (d36) and 48 h (d48). The area-under-the-bacterial-kill curve was between 0–24 h (AUBKC24) and 0–48 h (AUBKC48).
Results: As the killing kinetics of both agents were the same for both MRSA strains, the data was combined and compared (table).

	MRSA (2 strains)			VISA (1 strain)		
	tela	teico	P	tela	teico	P
d12	−3.4±0.5	−2.8±0.2	p = 0.02	−2.0±0.4	0±0.4	p < 0.01
d24	−3.1±0.6	−2.9±0.2	p = 0.41	−2.7±0.2	1.0±0.4	p < 0.01
d36	−2.8±0.3	−2.6±0.3	p = 0.28	−3.1±0.6	1.2±0.3	p < 0.01
d48	−2.7±0.5	−1.3±1.4	p < 0.01	−2.8±0.9	1.0±0.3	p < 0.01
AUBKC24	22.7±10.1	42.0±2.9	p < 0.01	55.1±4.7	92.7±5.0	p < 0.01
AUBKC48	46.7±17.0	80.4±10.7	p < 0.01	79.9±10.5	216.2±13.9	p < 0.01

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

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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/12 h, 1000 mg/8 h, 2000 mg/12 h, 1500 mg/8 h and 1500 mg/6 h) and daptomycin (4, 6, 8 mg/Kg every 24 h). 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/8 h. However, in United Kingdom and in Spain, CFR > 90% is only achieved with the highest dose: 1500 mg/6 h. 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*

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Objective: To study the antibacterial activity (log10 reduction) and time to obtain bactericidal activity (time to obtain $\geqslant 3 \log_{10}$ – 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/l daptomycin or 65.7 mg/l 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 Ca2+ (by adding 100 mg/l of Ca2+ in media containing albumin and 50 mg/l in media without albumin) in order to obtain a physiological free Ca2+ concentration.
Results: MIC/MBC (mg/l) of daptomycin and vancomycin, T99.9% (h) and log10 initial inocula reduction at 12 h/24 h are shown in the Tables.

	Daptomycin							
MIC/MBC	1/1 Strain 1		1/2 Strain 2		2/2 Strain 3		2/4 Strain 4	
	T99.9%	12 h/24 h	T99.9%	12 h/24 h	T99.9%	12 h/24 h	T99.9%	12 h/24 h
Cmax-MH	2	5.8/5.9	1	6.0/6.0	1	6.0/6.0	2	6.0/6.0
Cmax-HAlb	3	5.5/5.6	2	6.0/6.0	2	6.0/6.0	3	5.2/5.9

	Vancomycin							
MIC/MBC	0.5/16 Strain 1		1/32 Strain 2		2/32 Strain 3		1/32 Strain 4	
	T99.9%	12 h/24 h	T99.9%	12 h/24 h	T99.9%	12 h/24 h	T99.9%	12 h/24 h
Cmax-MH	>24	0.1/0.4	>24	0.1/1.3	>24	0.1/1.0	>24	0.1/1.5
Cmax-HAlb	>24	0.1/0.9	>24	0.3/1.8	>24	0.2/1.3	>24	0.2/1.1

Conclusions: Daptomycin Cmax exhibited rapid ($\leqslant$3 h) bactericidal activity while vancomycin did not achieve bactericidal activity along 24 h 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

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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 = 0.981) 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 VAN PB is more consistent with healthy volunteer data (PB $\approx$ 30%) than to the limited patient data (PB $\approx$ 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

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Objectives: From April 2008 to August 2008, many cases of hepatitis A were notified in Korea. The aim of the present study was 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

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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 patomorfological changes showing reliable intrahepatic cholestasis in VHA, clinical manifested cases are single and found mostly in adult patients.
Material and Methods: The study includs 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 gipsy residential districts of Plovdiv – 2006 y. Analyzed are eight parameters characterising cholestasis syndrome: max

T BIL, max ALT, ALP, GGT, lack of urobilinogen in urine, liver sonography control, stay in the clinic and age.
Results: In 14 patients VHA evinces cholestasis component and 3 cases are with cholestasis form of the disease. These are 2.07 percent of the surveyed contingent. Of them – a young man of 17 years, eight women and eight men aged between 27 and 62 years, with an average hospital stay – 49.1±11.5 days, as the longest stay – 75 days, is of a 27 years old man. Hyperbilirubinaemia is higher in the heat of the disease, average – 356.6 mkmol/l, and detained more than 150mkmol/l over a month. The values of T BIL in the discharge of the patients are at 2.5 times above normal. Ten patients show transaminase activity over 1400U/l, average – 2011U/l, while one patient reached level of 4923U/l that lessened after hospital stay of 51 days. In 14 patients the investigated acute icteric form of the disease run expressed with distinct cytolysis. Against this background are the symptoms of cholestasis syndrome: itching, acholic stools, the disappearance of urobilinogen in urine, increased ALP and GGT.
Conclusion: VHA may manifest with cholestasis syndrome as 25 percent of these cases are severe form of disease and 75 percent – medium. This is confirmed by raised transaminase activity, higher T BIL, expressed adinamiya, intoxication, vomiting, headache, dizziness, objectified by the score system, we have created.

P1992 Contemporary epidemiological characteristics of viral hepatitis A in Plovdiv region, Bulgaria

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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-HAVt. 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-HAVt are as follows: in the group of people living under bad sanitary–hygienic conditions, the examined persons are positive of anti-HAVt 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 immunoprevention 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

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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

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Introduction: Outbreaks of hepatitis A have been recently observed worldwide, with special attention focused on homosexual men, i.v. 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 22–56 y, 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 HAV hepatitis, 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.

P1995 Medical nursing students' knowledge and attitudes toward viral hepatitis in Turkey: a multi-centre cross-sectional study

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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 of hepatitis A virus (HAV) and HBV infection in Turkey.
Methods: This cross-sectional study was conducted on third and fourth-year nursing students in 14 nursing schools 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±1.3. The mean knowledge score of students was 23.7±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). 56.8% of the students rated their own knowledge level of viral hepatitis as intermediate and 29.9% of them rated as good. Knowledge sources 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.

P1996 Chronic hepatitis E in human immunodeficiency virus-infected patients living in south-eastern France

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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/mm^3 (mean, 27/mm^3), 69 were recently diagnosed (in 2006) for their HIV infection (mean CD4cc, 386/mm^3), 31 had liver cirrhosis (mean CD4cc, 375/mm^3), and 17 were infected with HIV-2 (mean CD4cc, 481/mm^3).
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/mm^3, 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/mm^3 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 aetiologic agent of hepatitis in HIV-infected individuals and might cause chronic infection in this population. HEV RNA and IgM anti-HEV Ab testings should be performed for reliable diagnosis of HEV infection.

P1997 Acute liver failure due to HEV in a long-term oral contraceptive treated patient

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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 inmuno-enzymatic assay and confirmed by inmunoblot, 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 toxics or drugs was oral contraceptives during last 20 years. Patient got better after admission taking routine antiencephalopathy 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.

P1998 Prevalence of HBV, HCV and HIV infections among obstetrics/gynaecology patients

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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/Gynaecology of our hospital.
Materials and Methods: The study included all patients admitted to the Department of Obstetrics/Gynaecology 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 a Microparticle Enzyme Immunoassay (AxSYM, Abbott), whereas the Inno-Lia (Innogenetics) and NewLavBlot (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 anti-HBc titers (anti-HBc negative/anti-HBs positive). Chronically infected patients were all HBeAg 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/Gynaecology 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 HBeAg negative/anti-HBe positive pattern among chronically infected patients indicates a low risk of vertical perinatal transmission in this population.

P1999 Molecular epidemiology of hepatitis B virus infection among chronic carriers in Greece, 2000–2007

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Objectives: Hepatitis B virus (HBV) infection is a global health problem affecting >1/3 of the world's population. Greece is considered an intermediate endemicity 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). Virologic 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 HBeAg(+), 86/136 (63%) were anti-HBe(+), and 4/136 (3%) were HBeAg(+)/antiHBe(+). Mean ALT was 238 IU/L, and HBV-DNA levels ranged from 8.2×10^5–11.51×10^7 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.

P2000 Prevalence of sexually transmitted diseases among female patients presenting with hepatitis B virus and human immunodeficiency virus infections in a Nigerian teaching hospital

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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 human immunodeficiency (HIV)-positive female patients who had been found to also have HBV infection 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-occuring 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; $p = 0.0002$).
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.

P2001 Occult hepatitis B infection in Tunisian pregnant women

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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 123 women with anti-HBc isolated marker, anti-HBs concentration varied between 5 and 10 mUI/ml in 19 women. Detection of HBV-DNA was performed in 98 cases (with anti-HBs lower than 5 mUI/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

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Introduction: Nearly 400 million people worldwide suffer from chronic hepatitis B virus (CHB) infection. HBV strains can be classified into eight genotypes, designed A-H with distinct geographical distribution. Seven 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 baseline viral load $1.7\,10^7$ 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%). Coinfection 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 Telvibudine and N236T and A181V/T which confer resistance to Adefovir in 5(3.5%) of cases. Mutations associated with Entecavir or Tenofovir were not selectioned 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

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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 (Diapro-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 was3.5 ± 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 seroprevalance of hepatitis D in this study at compare previous study in Iran is nearly equal.

P2004 Prevelance of blood-borne viruses amongst antenatal clinic patients and blood donors in a tertiary referral hospital in Oman

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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 MSExcel 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 by relatives are 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

	Antenatal clinic	Blood donors
HBV		
Total patients	3142	6762
Total tests	7002	10100
Age range at first visit (yrs)	12–55	17–65
Mean age at first visit (yrs)	28.8	28.1
HBsAg positive patients	222	190
Prevalence rate (%)	7.07	2.81
Hepatitis B carriers, n (%)		
high infectivity	4 (0.12)	14 (0.21)
low infectivity	208 (6.61)	169 (2.5)
HBeAg and anti-BeAg antibody negative, n (%)	10 (0.34)	7 (0.1)
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

P2005 Atypical serological pattern in chronic hepatitis B virus infection: a real problem or a sensitivity default of the analytical kit?

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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: From 320 cases of chronic hepatitis and cirrhosis, 44 patients (13.7%) were selected as reaching our criteria. The sera were provided by 2 hospitals in 2007 in order to select samples for DNA/HBV genotyping analysis.

Measurements of HBsAg, anti-HBcAb, in paralel with HBeAg, anti-HBeAb were done using commercially available ELISA kit from DiaSorin (ETI-AB-COREK-PLUS – producer A). The discordant results for HBcAb obtained after first set of determination imposed the new tests using ELISA kits from Biomérieux (Hepanostika anti-HBc Uni-Form – producer B) and Orgenics (ImmunoLISA HBc Ab – producer C).

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 cutt-off values and we can consider the results as "low postive" and "echivocal" respectively. The lack of HBeAg and/or HBeAb 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 neccesary. 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 immunsupression; a future testing using sequence analysis for pre-core/core region of DNA/HBV is recommended.

P2006 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

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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 HBsAg, Bio Meriux, France). Anti-HBc and Anti-Hbs positive cases were omitted. We used Procleix ultrio (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 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-HBs(–)] 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.

P2007 Twenty-year follow-up of vaccination against hepatitis B in patients with chronic renal failure

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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 biannual thereafter. Samples were tested by ELISA methods for HBsAg, anti-HBs and anti-HBc. The patients without protective anti-HBs level after basic 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 350 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.
Grant support: IGA Ministry of Health, Czech Republic, NR/9257–3/2007.

P2008 Hepatitis B nucleic acid testing of healthy blood donors in Finland

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Objectives: Finnish Red Cross Blood Service (FRC-BS) started hepatitis B nucleic acid testing (HBV NAT) of all blood donations in 2008. Earlier, only hepatitis B surface antigen (HBsAg) was tested in FRC-BS blood donations. Annual detection of 1–2 cases of low grade HBV DNAemia without HBsAg (occult HBV infection) was anticipated, based on published European blood donor surveys.
Methods: Each blood donation was tested with the Ultrio Kit for the detection of HIV-1 RNA, HCV RNA and HBV DNA (Gen-Probe, Chiron), with a sensitivity of 13.48 IU/ml for HBV-DNA. Reactive samples were confirmed with the HBV Discriminatory Probe Reagent (Gen-Probe, Chiron). All samples were tested for HBsAg (Bio-Rad Monolisa HBsAg Ultra). Samples with positive HBV DNA or HBsAg result were additionally tested for hepatitis B core antibody (HBcAb, Abbott AxSYM CORE).
Results: During 2008, altogether around 270,000 donations and roughly 160,000 individuals were tested for HBV DNA. One donor with HBV DNA positivity without HBsAg was detected, the donor was positive for HBcAb and had a very low level of antibodies against hepatitis B surface antigen (HBsAb, 2 IU/L). Two previously HBsAg negative donors were found positive for HBsAg and HBV DNA. One donor cleared both HBV DNA and HBsAg within few weeks, with introduction of HBcAb. The other showed clearance of HBsAg and HBV DNA but no introduction of HBcAb.
Conclusion: With HBV NAT testing, one occult HBV case was detected during one year, as expected. The number of HBV positive blood donors was higher than in previous years, but there was no statistical change in the prevalence of HBV markers (HBsAg, HBV DNA). The prevalence rate of HBV carrier status in new donors was 0.22 per 100 000, only 1/20 of the rate reported by the National Infectious Diseases Register for the whole population. The incidence of HBV in blood donors was exceptionally high in 2008, 1.57 per 100,000, over twice as high as reported in the National Infectious Diseases Register. Since 1990 the incidence of HBV in blood donors has varied from zero to over 3/100,000. As there has been seven years since the latest HBV seroconversion in a blood donor, the high incidence rate in 2008 might suggest that HBV is reemerging in the Finnish population.

P2009 Evaluation of anti-HBs titre in surgeons vaccinated for HBV

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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 41who 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 weakly 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

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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

	HBsAg (log_{10} IU/mL), mean±SD (N)		
	Responder[a]	Non-responder	P-value[b]
Baseline	4.20±0.70 (61)	4.45±0.48 (71)	0.0204
Decline from baseline to			
week 12	0.19±0.52 (36)	0.08±0.43 (49)	0.2854
week 24	0.69±1.14 (52)	0.12±0.66 (60)	0.0052
week 48	1.01±1.27 (61)	0.28±0.64 (68)	0.0005
week 72	0.83±1.23 (58)	0.23±0.52 (67)	0.0020

[a]Response: HBV DNA ⩽10,000 copies/mL 6 months post-trt.
[b]Wald 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 month post-treatment and clear HBsAg and could help optimise the management of PEGASYS® therapy in patients with HBeAg-negative chronic hepatitis B.

P2011 Lamivudine treatment for acute severe hepatitis B

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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 efficasy of lamivudine for the treatment of acute severe hepatitis B virus infection in imunocompetent 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 hepatocyte lysis. 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

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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 HBeAg 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 6 (accompanied by L180M and V173L in one case each). 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

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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 HVB 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 rt A181T.
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 25×10^6 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/24 h). During a one year period, viral load decreased but always remaining about $8–9\times10^4$ UI/mL. A year and a half later viral load increased to 7.4×10^5 UI/mL. Suspecting a failure due to resistant HBV, adefovir was changed to tenofovir (300 mg/24 h). 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

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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 potantial 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

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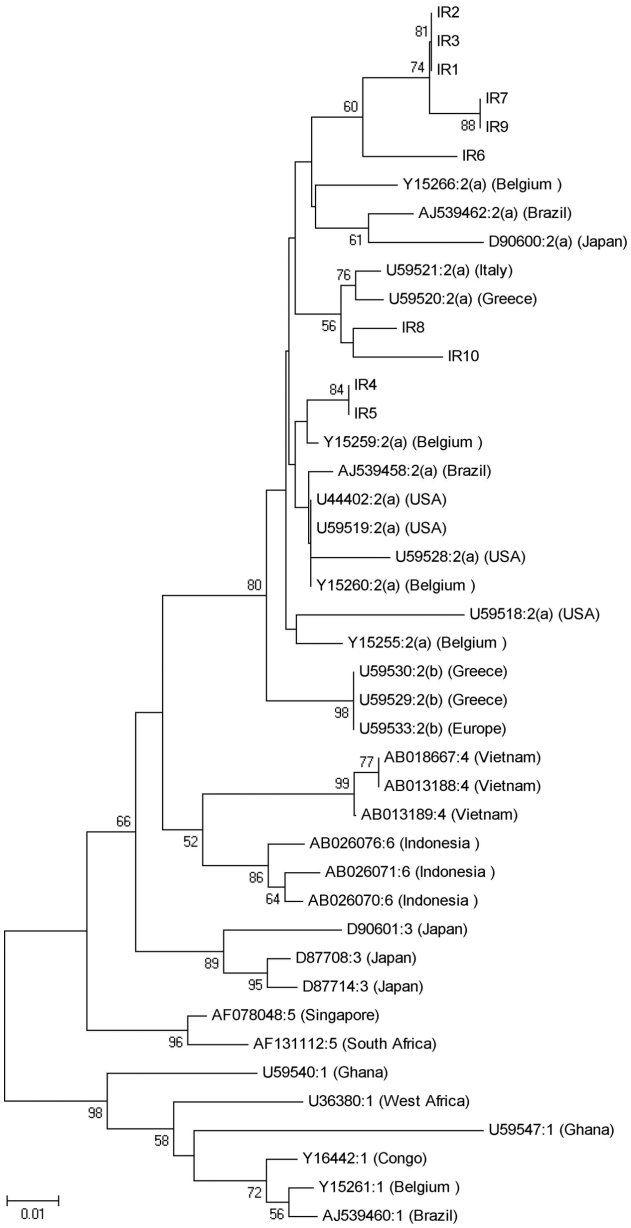
Objectives: Hepatitis G virus (HGV) infection is frequent in patients infected with the HIV due to similar transmission routes of these viruses. The aim of this study was to determine the rate of infection and genotypic characteristics of HGV in this population.
Methods: The presence of HGV RNA was determined 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

treatment with antiretroviral drugs. 66.7% of patients with HGV had a CD4+ cells count ⩾200 and 33.3% had a CD4+ cells count <200 cells/mm^3. Phylogenetic analysis revealed that all HGV isolates were genotype 2, and classified as subtype 2a.
Conclusion: HGV infection is relatively common in patients infected with HIV. The prevailing HGV genotype 2a in this study group concurred with reports from other parts of the Middle East.

P2016 Accidental blood exposures among medical residents in Paris, France

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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 operatory 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 personnels' experiences with sharps/needle-stick injuries and preventive measures

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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 personnels' 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 care clinics were interviewed. In our study the injury rate was %79.1; %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 ($P = 0.006$, $\chi^2 = 10.378$). 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

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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 immunublot 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

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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 exhibite dual anti-HCV and anti-HIV activities in vitro with little toxicity to the host cell lines.
Acknowledgement: This work is supported by NIH grant # 1-R01-GM-087738–01A1.

P2020 Evaluation of the results of the SEIMC External Quality Control Programme for HCV viral load

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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.

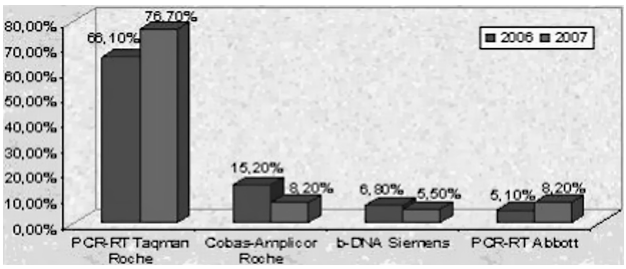

Figure 1.

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

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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 viraemic 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

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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, mir-30, mir-128, mir-196, mir-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

analysis of MxA, a well-characterised IFN type I-induced gene, was also performed as positive control.
Results: PBMC from patients with HCV express all examined miRNAs but their levels showed high variability (coefficient of variation >100%). In addition we observed that the overall expression of miRNAs was significantly different between patients with HCV and healthy subjects. When levels of the above miRNAs were measured 12 hours after the first injection of IFN, increases in expression levels of these miRNAs were observed in a percentage of patients ranging from 33.3% to 66.6% depending on the type of miRNA examined.
Conclusion: These findings suggest that miRNAs can be differentially induced by IFN treatment in HCV positive patients. Given the important role of miRNAs in defending the host against virus infection it is possible that such miRNAs may represent an important determinant of the clinical outcome of IFN therapy in HCV infection.

P2023 Acute hepatitis C and nosocomial transmission of hepatitis C virus: an emergent threat in the hospital setting?

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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: Seven patients had one or more nosocomial risk factors that were associated with several diagnostic and/or therapeutic procedures; three patients were iv drug abusers, one had recent dental surgery, one had a HCV-positive wife, the remaining one having no known risk factors. Seven patients had SR of AHC, three responded to PegIFN alpha 2b + ribavirin, and two relapsed to PegIFN alpha 2a + ribavirin, as shown in the table; the 13th patient had spontaneous biochemical normalisation with viral persistence.

Age	Risk factors	Genotype	Peak PCR	Peak ALT	Therapy	Outcome
23	IV drug abuse	1b	3.53×10^4	1,841	PegIFN alpha-2b	SVR
54	Colonoscopy, Herniorrhaphy	1b	1.8×10^6	1,615	No	SR
73	Coronary angiography/plasty, Blood transfusion	2a/2c	1.26×10^5	969	PegIFN alpha-2b + Riba	SVR
58	Gastroscopy, Colonoscopy	2a/2c	7×10^5	1,108	PegIFN alpha-2b + Riba	SVR
46	Dental surgery	1b	1.17×10^5	749	No	SR
48	Blood transfusion, Haemodialysis	1a	1.49×10^4	138	No	SR
61	Coronariography	2a/2c	4.18×10^6	1,175	PegIFN alpha-2a + Riba	Relapse
69	IV injection	1b	1.99×10^6	733	No	Viral persistence
67	ERCP, Cholecystectomy	1b	8.14×10^4	1,356	PegIFN alpha-2a + Riba	Relapse
66	HCV+ wife	1b	1.54×10^4	2,514	No	SR
30	IV drug abuse	1b	8.72×10^3	3,118	No	SR
35	None	3a	1.45×10^7	2,639	No	SR
30	IV drug abuse	1b	$>6.9\times10^7$	3,894	No	SR

ERCP, endoscopic retrograde cholangiopancreatography; ALT, alanine aminotransferase; SVR, sustained virological response.

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.

P2024 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

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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 1.23×10^7 copies/ml and only 28 (27.5%) had detectable HIV viral load. Most subjects were receiving HAART (89%) and mean CD4 cells was 479 mm^3. The CD4+ nadir was 220 per mm^3. 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 ($\leqslant$7 kPa) in 33%, F2 (7.1–9.4 kPa) in 20%, F3 (9.5–12 kPa) in 11% and F4 (>12 kPa) in 35%. As compared with those without significant fibrosis, absolute, percentage and nadir of CD4+ cells, platelets count, cholesterol level and protrombine activity were lower in patients with significant fibrosis ($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 HCV and HIV who are not receiving treatment for HCV have more hepatic fibrosis if they are men, if are co infected with HBV, if have genotype 1 and if are smokers, heavy alcohol drinkers and cannabis consumer. 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.

P2025 Genetic diversity of hepatitis C virus among Bulgarian injecting drug users with hepatitis C

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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 Hepatitis C virus (HCV) Test, version 2.0 (Roche Molecular Systems, Inc, Branchburg, MJ, 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

by RT-PCR for the presence of HCV RNA. One hundred and fifteen (78.2%) from the anti-HCV positive samples were positive for HCV RNA. The genotype was determined in 113 samples and 2 sera positive for HCV RNA couldn't be genotyped.
Genotype 1a was detected in 2 (1.7%) and genotype 1b in 72 (63.7%) of the samples. In only two cases with samples from genotype 1, the subgenotype couldn't be determined. Genotype 3a was present in 37 (32.7%) of the samples. No other genotypes were available in tested samples.
Conclusions: These results demonstrate that the genotype 1b of HCV is the most prevalent in Bulgarian drug users, but 3a genotype can be often detected also. These two genotypes (1b, 3a) of HCV are predominant in this high risk group.

P2026 Development of a low-cost approach for quantitation and genotyping of hepatitis C virus

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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 coefficient of variation (CV) of the standard curve was, on average $\geqslant$0.99. 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-alpha 2a 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 = 0.875 $P < 0.001$).
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.

P2027 Oxidative stress and antioxidant defence in patients with chronic hepatitis B and C

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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 13.8, $p < 0.05$; 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.

P2028 APRI Index, HCV genotype and HCV RNA as predictors of early viral response in hepatitis C patients

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Background: Absence of therapeutic response in hepatitis C patients is multifactorial; genotype 1, viral load major than 800,000 UI/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 α-2b pegylated and ribavirin and reviewed retrospectively clinical charts of patients with the same diagnosis from January 2004 to June 2006. The treatment with Interferon α-2b pegylated 1.5 μg/kg/weeks and ribavirin was adjusted (>75 kg; 1200 and <75 kg; 1000 mg). 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 HVC RNA >800,000 UI/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.

P2029 The efficacy and adverse events of standard interferon (alpha 2a or 2b) plus ribavirin versus pegylated interferon (alpha 2a or 2b) plus ribavirin

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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 α-2a or 2b (3 MIU tiw) plus ribavirin (1000–1200 mg qd) for 52 weeks (Group A) and 42 patients received peginterferon α-2b (1.5 μg/kg subcutaneously weekly) or Pegylated interferon α-2a (140 μg or 180 μg subcutaneously weekly) plus ribavirin (1000–1200 mg qd) for 52 weeks (Group B).

Results: Baseline ALT levels were 79.05±53.82 in group A; 106.5±79.55 in group B, and fibrosis scores were 1.75±1.16 in group A; 2±1.31 in group B. In Group A genotype 1 was 91%; genotype 3a was 9% and in group B genotype 1 was 82%; genotype 3a was 10% and genotype 1+4 was 8%. End of treatment biochemical response was 89% in Group A and 78% in group B, sustained virologic response 63% in group A and 73% in group B, and similar relapse rates of 17% in both groups. The percentages of adverse events were 92% and 97%, respectively.
The most common adverse event was asthenia. Standard interferon plus ribavirin was discontinued in 4 of 56; peginterferon plus ribavirin was discontinued in 5 of 42.
Conclusion: In conclusion, no significant difference on efficacy and adverse events between the two treatment schedules was observed in this study. Both combination therapeutic regimens are effective in inducing a sustained virologic response.

Emerging or re-emerging infections

P2030 Evaluation of the anthrax cases who were admitted to our hospital in 2008

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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

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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 contact with irrigation ditches, 2 were bricklayers and in two cases an international travel was associated. The more frequent symptoms were: fever (100%), artromyalgia (56%), dispnea (32%), Shock (20%), Abdominal pain(36%), neurologic involvement(15%) and jaundice(24%). The laboratory data showed: 16 patients renal impairment(43% precised dialysis), 70% rises in transaminase levels and 11% elevated levels of alkaline phospatase. 24% presented with a total bilirrubin count more than 10 mg/dL. Elevation of CPK occurred in 25 patients and the levels of rabdomiolisis 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 Doxicycline (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

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Objectives: We have used polymyxins since 1990 s 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 10^5 CFU/ml at baseline with the maximum, clinically achievable, unbound concentration (mg/L) of PB (2), (R)rifampicin (2), (M)meropenem (64), (C)cefepime (200) and (T)tigecycline (2) alone and in combination against the 2 isolates.

Table 1: MIC (mg/L) panel of XDR AB 1 and XDR AB 2

Antibiotic	XDR AB 1	XDR AB 2
Ampicillin/Sulbactam	32	32
Ciprofloxacin	⩾16	⩾16
Gentamicin	⩾64	⩾64
Imipenem	⩾64	32
Meropenem	128	64
Aztreonam	⩾128	64
Piperacillin/Tazobactam	⩾256	⩾256
Polymyxin B	64	32
Tigecycline	4	4
Ceftazidime	⩾128	⩾128
Amikacin	⩾2048	⩾2048
Cefepime	128	128
Rifampicin	2	4

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

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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 1×10^5 to 5×10^5 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 a 3-drug combination AMP (SGH), LRP(NUH), ALRP(TTSH), ALMP(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, ALMP, 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

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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 patterns.
Materials and Methods: In 2008, 6 cases of *Nocardia* infection occurred in our Hospital. Five patients were immunocompromised: 4 of them presented with pulmonary infection and one with pulmonary infiltrates and disseminated cutaneous nodular disease. A sixth patient presented with respiratory infection and no sign of immune deficiency. Five *Nocardia* strains were isolated from respiratory samples (BAL and sputum) and one from pus taken by FNA. All specimens were examined by Gram stain, inoculated onto blood agar and incubated for at least 7 days at 35ºC. Phenotype-based identification followed standard routine laboratory protocols. The susceptibility profile of all strains to 12 antibiotics was determined using Etest (AB Biodisk, Sweden), according to the CLSI standard method. All isolates were subjected to further identification by16 s rRNA sequencing and phylogenetic analysis.
Results: Based on a similarity of >99% to the closest relative 16S rRNA sequences, 5 strains were identified as *N. cyriacigeorgica*. The sixth isolate, a slow growing and phenotypically different strain, was *N. abscessus* derived from an immunocompetent patient with lung infiltrates. The 16S rRNA sequences were clearly distinct from sequences available in GenBank for *N. asteroides*, supported by high bootstrap values.
N. cyriacigeorgica strains were resistant to β-lactams, ciprofloxacin, piperacillin/tazobactam and clarithromycin but susceptible to imipenem, linezolide, moxifloxacin and amikacin. The *N. abscessus* isolate was resistant only to ciprofloxacin and piperacillin/tazobactam.
Conclusions: This study indicates the high incidence of *Nocardia cyriacigeorgica* in the clinical setting, showing major association with lung infections. Molecular methods have improved accurate diagnosis, resulting to the emergence of novel species. To our knowledge, these are one of the few reports of disseminated *N. cyriacigeorgica* infections in Europe.

P2035 Pertussis remains a health problem

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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

(54%). In addition antibodies were detected in the serum of 45 (78.9%) adults of the second group and in 23 (69.7%) adults of the third group. **Conclusions:** Despite the obligatory vaccination, pertussis continues to be an infectious disease of all ages. Increased prevalence of the disease was observed with increasing age. Pertussis is often responsible for the infections of the close contacts (family environment) of the patient. The chronic cough of the patients is often due to pertussis. Pertussis diagnosis remains difficult in case of neonates and small children.

P2036 Pigs as a source for toxigenic *Corynebacterium ulcerans* in diphtheria-like disease

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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 afflicted 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 cardiomyopathia.
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 neeeded.
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 afflicted by binasal discharge. Here, we report the first case of severe *C. ulcerans* diphtheria-like disease after pig contact.

P2037 Detection and enumeration of *Clostridium difficile* in retail meat

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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, toxinotyping 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.

Source	Ribotype	Toxinotype	Toxin genes	n (%)
Ground beef	078	V	A+, B+, CDT+	10 (71)
	027	III	A+, B+, CDT+	1 (7.1)
	C	IX	A+, B+, CDT+	1 (7.1)
	Not tested			2 (15)
Ground pork	078	V	A+, B+, CDT+	9 (64)
	027	III	A+, B+, CDT+	1 (7.1)
	C	IX	A+, B+, CDT+	1 (7.1)
	Y	III	A+, B+, CDT+	1 (7.1)

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.

P2038 Clinical characteristics of infections with *Clostridium difficile* ribotype 027 versus other ribotypes: data from prospective surveillance in Belgium

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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.

Table: Comparison of *C. difficile* infections caused by *C. difficile* ribotype 027, and other ribotypes

	Ribotype 027 (N = 211)		Other ribotypes (N = 769)		Ratio of proportions	P (χ^2)
	n	%	n	%		
Female gender	136	64%	409	53%	1.2	0.00
Age ⩾80	121	57%	323	42%	1.4	0.00
Hospital-associated CDI (⩾2 days after admission)	130	62%	486	63%	1.0	0.8
Underlying condition fulminant or rapidly fatal	27	13%	95	12%	1.0	0.7
Death	48	23%	150	20%	1.2	0.3
CDI-related death	33	16%	85	11%	1.4	0.06
Median time from infection (or hospital admission for imported cases) to discharge, days	20		19		ns*	
Median time from infection to CDI-related death, days (N = 114 CDI-related deaths)	14		15		ns*	

*Mann-Whitney test – comparison of medians.

Results: Laboratory data were available for 980 episodes of *C. difficile* infections (CDI) which occurred between July 1, 2006, and June 30,

2008 across 80 Belgian hospitals; 211/980 (21.5%) strains belonged to the ribotype 027. Simple univariate comparisons can be found in the table.
Using logistic regression, and taking into account the clustering effect of hospitals, we modelled the probability of CDI-related death (CDI as direct or indirect cause of death, dependant variable) using as covariate age (<80 vs ⩾80), sex, severity of underlying condition (not fatal, ultimately fatal, fulminant or rapidly fatal), and the ribotype (ribo 027 vs other). We also added the time from infection to discharge (1–7 days, 8–14 days, ⩾14 days) in the model to account for the time-dependant nature of the observations.
Age over 80 (odds ratio, OR 1.6, p =0.01) and the severity of underlying condition, but not sex, were independent predictors of CDI-related death. Infection with ribotype 027 was associated with an OR of 1.9 (p = 0.041).
Conclusions: In this large prospective study, patient data and laboratory data were collected independently and are therefore free from any differential bias. CDI with ribotype 027 were no more frequent in hospital-associated cases than in imported cases, and resulted only in slightly higher length of stay after infection, but the odds to die from a ribotype 027 CDI were almost double than the odds of dying from CDI with another ribotype.

P2039 *Clostridium difficile* in dogs and the home environment: prevalence and risk factors

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Objectives: A cross-sectional survey was conducted to determine the prevalence of *C. difficile* shedding in dogs and the home environment, compare ribotypes, and identify risk factors for *C. difficile* shedding.
Methods: Households containing dogs were recruited in southern Ontario. Canine faecal samples were collected for 5 consecutive days. Enrichment culture for *C. difficile* was performed and isolates were characterised by ribotyping, toxinotyping and toxin gene PCR. Nine household sites were also tested (table) and a questionnaire was administered.
Results: 84 households and 137 dogs were enrolled. *C. difficile* was isolated from 14/137 (10%) dogs, but 13 (93%) dogs were only positive on 1/5 samples. Dogs living with an immunocompromised person were 8× as likely (p = 0.02) to shed *C. difficile* than other dogs while dogs allowed to run freely in a park were 3× less likely (p = 0.04) to shed *C. difficile*. 10 (71%) canine isolates were toxigenic. 5 different ribotypes were present among toxigenic strains, all of which have been found in people in Ontario. The most common was a toxinotype (TT) 0 strain that is the most common strain in hospitalised humans in the province. Two other TT0 strains were identified, plus a TT XII strain and a binary toxin-positive TT IX strain. 77 *C. difficile* isolates, 62 (81%) toxigenic, were found in 26 (31%) households, with 1 to 4 positive samples per household (table). Ribotype 027 was most common (13%), followed by ribotype 078 and a TT0 ribotype (8% each). *C. difficile* was isolated concurrently from dogs and the environment in 4 households, but in all cases canine and environmental ribotypes were different.

Site	Prevalence	Site	Prevalence
Toilet	9/83 (11%)	Dog eating area	4/84 (4.8%)
Dog food bowl	6/84 (7.1%)	Kitchen sink taps	4/84 (4.8%)
Refrigerator	6/84 (7.1%)	Main entryway	2/84 (2.4%)
Kitchen sink	6/84 (7.1%)	Vacuum bag contents	1/81 (1.2%)
Kitchen counter	4/84 (4.8%)		

Conclusions: Although the prevalence of *C. difficile* in dogs is low, the fact that all toxigenic strains are recognized human pathogens raises concern about interspecies transmission. The relationship between the presence of an immunocompromised individual in the home and *C. difficile* shedding by their dog supports this concern. The sporadic nature of isolation of *C. difficile* from dogs could represent intermittent shedding, test sensitivity or transient passage (as opposed to colonisation). The high prevalence of household contamination suggests that exposure to low levels of *C. difficile* may be a common event and the commonness of positive kitchen sites raises questions about food as the source of contamination. This study does not provide evidence that dogs are an important source of environmental *C. difficile* contamination.

P2040 Prevalence of *Clostridium difficile* in retail pork

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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 toxinotyping, 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 toxinotypes were identified. Three isolates were ribotype 027 and toxinotype III, with genes encoding toxins A, B and binary toxin, an 18 bp tcdC deletion and a truncating mutation in tcdC. One strain had a different ribotype from 027 but was toxinotype III, positive for all 3 toxin genes and had the same tcdC deletion and mutation as 027. One isolate was toxinotype XXVI and possessed genes for toxins A and B and an unaltered tcdC gene, while another was a toxinotype 0 strain possessing the same toxin gene characteristics. One isolate was non toxigenic. 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 implications 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.

P2041 *Clostridium difficile* colonisation in veal calves

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Objectives: There is increasing concern that *C. difficile* may be a zoonotic pathogen, and that food may be a source of infection. While *C. difficile* can be found in retail beef and veal, there is very little information available about *C. difficile* colonisation in cattle. The objective of this study was to longitudinally monitor *C. difficile* colonisation in veal calves.
Methods: 163 male Holstein-Friesen calves on one farm were enrolled. Calves were approximately 2 weeks of age at arrival, came from several different farms and were housed either individually or in groups in two separate pens. Calves are managed in an all-in/all-out system and treated with oral oxytetracycline after arrival. Faecal samples were obtained on day 1 or 2 after arrival and 6 days later. Further sampling is ongoing. Selective culture for *C. difficile* was performed, and isolates were characterised using standard methods.
Results: *C. difficile* was isolated from 22/70 (34%) individually housed calves at the first sampling period and 42/69 (61%) 6 days later (Pfts;< 0.001). Similarly, *C. difficile* was isolated from 31/93 (33%)

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 *C. difficile* in diarrhoea was not evaluated because of the high overall prevalence of diarrhoea.

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.

P2042 First detection of *Clostridium difficile* ribotype 027 in Bochum, Germany, confirmed by slpA sequencing

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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 occured 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.

P2043 Leptospirosis: an emerging disease in rural areas in Greece

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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 failutre, it was present in all cases examined.

P2044 Clinical spectrum and microbiological features of nocardial infection in Crete, Greece: a seven-year experience

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Objective: To assess the incidence, species distribution, antimicrobial susceptibility, predisposing factors, treatment and outcome of nocardial infection over a 7-year period in a tertiary care hospital.

Methods: We reviewed the clinical and laboratory records of 20 patients with nocardial infection diagnosed at the University Hospital of Heraklion, Crete, Greece, between 1996 and 2002.

Results: All patients were adults. The male to female ratio was 3:1. The mean age was 57 years (range 32–83 years). Thirteen patients (65%) had one or more underlying conditions predisposing to nocardiosis. The skin was involved in 14 cases, followed by the lung (five); one patient had disseminated disease and one bacteraemia. Four different species were identified: *N. brasiliensis* (n = 11), *N. otitidiscaviarum* (n = 4), *N. abscessus* (n = 3), and *N. farcinica* (n = 2). All isolates were sensitive to linezolid, moxifloxacin, and tigecycline, and 85% of them to trimethoprim-sulfamethoxazole. Treatment with trimethoprim-sulfamethoxazole (SXT), minocycline, imipenem with amikacin, linezolid alone or in combination with quinolones was given to all 20 patients. A cure was observed in 18 of them, while two died of nocardiosis.

Conclusion: Nocardiosis is a rare but sometimes lifethreatening opportunistic infection, complicating immunosuppression. Molecular methods can contribute to rapid and accurate diagnosis. Early identification and antibiotic susceptibility testing of *Nocardia* isolates are necessary for prescribing the appropriate treatment. *Nocardia brasiliensis* was the most common species found in our study and SXT was the most frequently administered treatment.

P2045 Hepatitis E virus detection in wild boar liver samples from south-eastern France

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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 200 µl 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 others, 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

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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 samples 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 autoptic 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 parvoviruses from animals in Hong Kong discovered two novel parvoviruses closely related to human parvovirus 4

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Background: Parvoviruses are a family of viral pathogens with a wide animal tropism. However, within the vertebrate specific sub-family Parvovirinae, only a few members were known to be associated with human disease, these are parvovirus B19, adeno-associated viruses, and more recently, the human bocaviruses and human parvovirus 4 (PARV4). PARV4 is an interesting novel human parvovirus since it is found to have less than 30% amino acid similarity to other parvoviruses forming a distinct branch in the phylogeny of the Parvovirinae 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 parvovirus.
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 PARV-4 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 PARV-4 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 parvoviruses that are closely related to PARV4 where the three formed a distinct cluster among other parvoviruses.
Conclusion: Two novel animal parvoviruses closely related to PARV4 were found. From the genomic organisation and phylogenetic analysis, we propose that these two novel parvoviruses, together with PARV4, to form a separate genus.

P2048 Crimean-Congo haemorrhagic fever among healthcare workers in Turkey

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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 the all HCWs.

P2049 Crimean-Congo haemorrhagic fever in Turkey

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Objective: Crimean-Congo haemorrhagic fever(C-CHF) is severe viral disease afflecting multiple organ systems. It is caused by infection with

a member of the genus Nairovirus in the family Bunyaviridiae. C-CHF has been found Eastern Europe, Central Asia, the Middle East, China and Indian subcontinent. Many cases have been reported from Turkey. We herein reported 8 cases with Crimean-Congo haemorrhagic fever.
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 patiens), 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, trombocytopenia 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, >530) 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 immunassay (ELISA) in four patients. Serum sample of one patient was positive by RT-PCR. Three serum samples were positive for both ELISA and viral genom 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.

P2050 Crimean-Congo haemorrhagic fever in Kosova, 1989–2008

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Crimean-Congo Haemorrhagic Fever (CCHF) is acute viral zoonossis that appears after tick bit 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 epidemiological, 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 bit. Human transmission is found in 21.2% cases inside family, and in 2.42% 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%), haematuria (29.2%), metrorrhagia (34.5%), liver disorders like hepatitis and mild renal disorders. Specific manifestations in our cases are: otorrhagia (2.03%), Herpes Febrilis (6.6%), haematoperitoneum (13.2%), pleuritis haemorrhagica (5.6%), pericarditis haemorrhagica (8.6%) and thrombocitosis 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.

P2051 Evaluation of risk factors for fatality in patients with Crimean-Congo haemorrhagic fever

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Objectives: Crimean-Congo Haemorrhagic Fever (CCHF) is a fatal viral infection caused by the CCHF virus. Fatality rate of the disease has been reported as 5–50%. In this study we aimed to evaluate the risk factors for fatality in patients with CCHF hospitalised in Ankara Training and Research Hospital, between 2006 and 2008.
Methods: All CCHF patients admitted to our clinic between 2006 and 2008 were evaluated. Serum samples were analysed with immunological (specific ELISA IgM and IgG) and molecular (RT-PCR) assays for the confirmation of the disease. All patients with positive IgM antibodies and/or PCR for CCHF virus in blood were included to the study. To determine the predictors of fatality among patients with CCHF, we compared epidemiological, clinical and laboratory findings of the fatal cases with survivors.
Results: Ninety-three confirmed CCHF patients were included in the study. Fifty-six (60.2%) of them were female, mean age was 48.4±17.7 years (14–83 years) and mean hospital stay was 7.9±3.0 days (1–18 days). Five patients were died (5.4%). The age, gender, the rate of tick bite, time from tick bite to admission to the hospital, hospital stay, initial complaints (fever, fatigue, nausea, vomiting, myalgia, abdominal pain, headache and skin eruption) were not significantly different between fatal and non-fatal cases ($p > 0.05$). The rates of haemorrhage, diarrhoea and confusion were higher in fatal cases compared with non-fatal cases ($p < 0.05$). Mean aspartate aminotransferase (AST), alanin aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatin phosphokinase (CK), and C-reactive protein (CRP) levels were higher in fatal cases than the non-fatal ones, activated partial thromboplastin time (aPTT) was longer and mean platelet counts were lower ($p < 0.05$). There was no differences between ribavirin and steroid therapies, and fresh frozen plasma, platelet and erythrocyte infusions between the two groups ($p > 0.05$). By multivariate analysis only elevated serum ALT, AST, LDH levels and prolongation of aPTT were independently risk factors associated with fatality.
Conclusions: In this study we found serum AST, ALT, LDH and aPTT values as predictors for fatality among patients with CCHF. We suggest that for patients who had these abnormal laboratory findings, physicians should be aware of high fatality risk.

P2052 Clinical findings and laboratory data in Crimean-Congo haemorrhagic fever as a re-emerging diseases

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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 (Hialoma). 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%), Heamaturia in 20 cases (62%), Echymosis in 10 cases (32%), Icter 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

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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 inhabitating 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* sp. 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 *Anaplasma* 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 freqent 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. 4/39, $p < 0.05$).
Conclusion: The results show frequent coinfections 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

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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 rRNA.
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 underlying lung disease or immunosuppression. Mean age of Pneumocystis carriers was higher than mean age of noncarriers (66.6±17.3 vs. 51.2±21.5, $p = 0.01$). No differences were detected due to sex and smoking habit between carriers and noncarriers.
Conclusions: 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. Inmunocompetent carriers in community ecosystems may present a public health issue that merits further research.
Supported by CIBER de Epidemiología y Salud Pública and ERA-NET Pneumocystis-PathoGenoMics.

P2055 *Giardia* and *Cryptosporidium* in Finland – who gets reported?

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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 peacetime, 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

traveller's diseases which might have led to underdetection of these parasites in Finland.

P2056 Infectious diseases of immigrants – present screening systems in Finland

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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 practises 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-pen 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, HIV and syphilis. 52% of the respondents are satisfied with existing instructions to conduct screening although 69% of the respondents would 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 1: Testing of different infectious diseases from immigrants

Disease	Tests	% of respondents who had tested this from immigrants
Tuberculosis	Chest radiograph	60
	Tuberculin skin test	30
Hepatitis B	Surface antigen (HBsAg)	82
	Core antibody (HBcAb)	56
Hepatitis C	Hepatitis C Antibody (HCV-Ab)	66
HIV	HIV antibody	75
Syphilis	Cardiolipin	63
	Treponema Pallidum Haemagglutination Assay (TPHA)	33
	Treponema Pallidum antibody (Trpa-Ab)	22

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

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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 10^1 CFU/ml and 10^6 CFU/ml, respectively, and each of the 21 isolates spiked into stools at 3 concentrations (10^6, 10^3, 10^1 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-disk synergy test. Mean sensitivity (SEN) and specificity (SPEC), and confidence intervals (CIs) 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.

Table. Mean sensitivities and specificities of media for ESBL-En detection

Media for ESBL-En detection	24 hours				48 hours			
	Sensitivity		Specificity		Sensitivity		Specificity	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Reader response aggregated								
EbSA* (Alpha-omega, BE)	79.6%	75.3, 83.3	99.6%	94.6, 100.0	86.0%	82.6, 88.8	99.4%	91.8, 100.0
ChromID (bioMérieux, FR)	84.1%	80.2, 87.4	99.6%	94.7, 100.0	89.3%	86.3, 91.7	99.4%	91.9, 100.0
CHROMagar (CHROMagar Microbiology, FR)	77.5%	73.0, 81.4	96.2%	91.4, 98.4	84.4%	80.8, 87.5	94.1%	87.1, 97.4
Amber (AES Chemunex, FR)	54.4%	47.6, 61.0	74.0%	62.5, 83.0	65.3%	58.8, 71.2	64.4%	51.4, 75.6
Chromogenic ESBL (Oxoid, UK)	99.4%	97.6, 99.8	99.2%	95.0, 99.9	99.6%	98.5, 99.9	98.7%	92.3, 99.8
Reader response penalised								
ChromID (bioMérieux, FR)	75.9%	71.5, 79.7	99.6%	94.4, 100.0	81.8%	78.1, 85.0	99.5%	92.1, 100.0
CHROMagar (CHROMagar Microbiology, FR)	49.7%	44.8, 54.6	96.3%	91.5, 98.4	58.6%	53.7, 63.3	94.7%	88.3, 97.7
Amber (AES Chemunex, FR)	26.4%	22.2, 31.1	67.6%	57.2, 76.5	33.9%	29.0, 39.1	59.3%	48.4, 69.4
Chromogenic ESBL (Oxoid, UK)	82.2%	78.3, 85.6	98.1%	93.7, 99.4	86.9%	83.6, 89.5	97.3%	91.2, 99.2

*EbSA is a selective medium that does not differentiate the ESBL-En and thus the aggregated and penalised responses are the same.

P2058 A laboratory evaluation of chromogenic screening media for the detection of extended-spectrum β-lactamase producing bacteria

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Objectives: Since the 1980 s, 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

intensive and time consuming. The aim of this study was to examine the performance of two chromogenic screening media for the detection of ESBL-producing bacteria, ChromID™ ESBL (bioMérieux) and Brilliance™ ESBL Agar (Oxoid).
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 37ºC 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-ESBLs. Brilliance ESBL Agar correctly identified 22 of 23 ESBLs and 53 of 57 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

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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 eSwab were inoculated onto chromID™ ESBL and CTAZ. Plates were incubated at 35ºC 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 blaTEM, blaSHV and blaCTX-M genes. Isolates harbouring blaTEM and/ blaSHV 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 *Escherichia coli* (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.

P2060 Evaluation of a chromogenic bioMérieux chromID™ ESBL medium for screening and presumptive identification of extended-spectrum β-lactamase-producing Enterobacteriaceae from surveillance cultures

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Objectives: We evaluate a selective and differential chromogenic medium, chromID™ ESBL (bioMérieux, Marcy l'Etoile, France) which enables selective isolation and presumptive identification of extended spectrum β-lactamase-producing Enterobacteriaceae (ESBL) from clinical samples.
Methods: A total of 293 surveillance rectal swab samples were in parallel inoculated to chromID™ ESBL and MacConkey agar (MCK, Tec-Laim. Madrid. Spain) and incubated for 24–48 h. Pink-burgundy, green-blue and brown colonies on chromID™ ESBL were presumptively identified as ESBL-producing *Escherichia coli*, *Klebsiella/Enterobacter/Serratia/Citrobacter* group or Proteeae respectively. Any coloured colonies (pink-burgundy, green-blue or brown) and all oxidase-negative colonies on MCK were performed combined double disc method for ESBL confirmation and were also identified and tested susceptibility by the MicroScan system (Dade Behring).
Results: Overall, 95 Enterobacteriaceae isolates were recovered on 91 specimens (31.1% of the total specimens studied) by at least one of the two media. Eighty-four specimens were positive by both media and eight specimens were positive only by chromID™ ESBL (7.7% of all positive samples). The distribution of ESBL-producing microorganisms was as follows: 64 *E. coli*, 21 *Klebsiella pneumoniae*, 7 *Klebsiella oxytoca*, 1 *Citrobacter amalonaticus*, 1 *Enterobacter cloacae* and 1 *Enterobacter aerogenes*. The overall sensitivity, specificity, positive predictive value and negative predictive value of chromID™ ESBL were: 100.0%, 92.6%, 85.8%, and 100.0%. On chromID™ ESBL, a total of 18 non-ESBL-producing organisms presented the chromogenic appearance of ESBL-producers: *E. cloacae* (7), *E. coli* (4), *Klebsiella* spp. (2), *Pseudomonas aeruginosa* (2), *Serratia marcescens* (1), *Citrobacter freundii* (1), *Stenotrophomonas maltophilia* (1). Most of these false positive results were due to Enterobacteriaceae overproducing chromosomical cephalosporinase.
Conclusions: Our results showed that chromID™ ESBL is an appropriate medium for the screening and presumptive identification of ESBL-producing Enterobacteriaceae directly from surveillance specimens. The enhanced recovery and the easier detection of ESBL-producing Enterobacteriaceae using this medium would benefit the patients and would decrease costs associated to infections caused by ESBL-producing microorganisms.

P2061 Evaluation of ESBL producing *E. coli* and *Klebsiella pneumoniae* strains with EUCAST and CLSI breakpoints

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Objectives: The detection of *E. coli* and *Klebsiella* ESBL producing strains is of great epidemiological importance for hospitals. Usually ESBLs are suspected after the first susceptibility test with various 3rd gen cephalosporins, before a specific ESBL-test is performed. We analysed 6,318 *E. coli* and 1,513 *Klebsiella pneumoniae* strains including ESBL producing strains to see whether CLSI or EUCAST breakpoints give hints for ESBL production.
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. Copy strains are excluded. Quality control is mandatory. The BD PHOENIX system detects ESBL producing strains based on results fo a specific ESBL test and other 3rd gen cephaspoins present in the panel.

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 only one MIC step marked differences in the % of resistant strains were seen applying the respective brekpoints (75 vs. 28%). The difference with Piperacillin/Tazobactam was not as pronounced (about 20 to 30% resistance CLSI and EUCAST) Among the *Klebsiella pneumoniae* 137 strains (9.1%) were ESBL producers. The MIC distributions showed similar phenomena.
Conclusion: To detect ESBL producing strains of *E. coli* and *Klebsiella* with the first susceptibility test laboratories should test very low concentrations of 3rd gen 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 range of different β-lactams because of the different specificity of the β-lactamases.

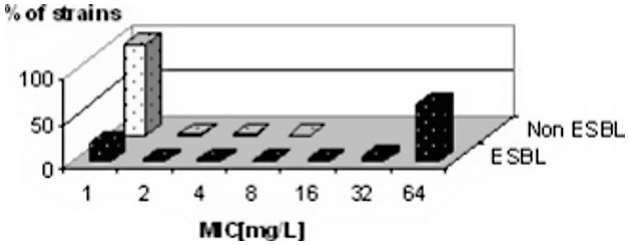

Figure: MIC distribution for ESBL and no-nESBL producing *E. coli*.

P2062 Clinical and molecular characteristics of phenotypically false-positive extended spectrum β-lactamases in Enterobacteriaceae in a low-endemicity setting

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Background: Detection of ESBL producing Enterobacteriaceae is difficult and requires testing with several screening antibiotics to optimise diagnostic sensitivity. False-positive ESBL reports from the microbiology laboratory (low specificity) result in overuse of broad-spectrum antibiotics and unnecessary infection control measures. The aim of our study was 1) to determine the rate of false-positive ESBL in a low-endemicity setting (<4% of strains) and 2) to identify clinical and phenotypic predictors for false-positive ESBL.
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. Cefpodoxime, ceftriaxone, ceftazidime and aztreonam as well as cefepime 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, whereof 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 (BLIC). OXA-1 strains had an elevated cefepime MIC, a positive ESBL confirmation test with cefepime, but were negative for all other screening compounds and also resistant to BLIC. *E. coli* strains were more likely to represent true ESBL than other species (OR 5, CI95 1.25–20). False-positive ESBL were more prevalent in patients with fatal comorbidities ($p = 0.014$) and cultures sites other sites 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.

P2063 Detection of extended-spectrum β-lactamase among Enterobacteriaceae using automated microbiology systems

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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 susceptility 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), *Serratia 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.

	E. coli		*K. oxytoca*		*K. pneumoniae*		*Enterobacter, Citrobacter*	
	Vitek 2	Walkaway	Vitek 2	Walkaway	Vitek 2	Walkaway	Vitek 2	Walkaway
Sensitivity	100.0	100.0	35.7	64.3	91.3	91.3	100.0	Nd
Specificity	100.0	100.0	100.0	0.0	100.0	83.3	44.4	Nd
NPV	100.0	100.0	18.2	50.0	75.0	71.4	100.0	Nd
PPV	100.0	100.0	100.0	81.8	100.0	95.5	52.4	Nd

P2064 Detection of AmpC enzymes and the prevalence of plasmid-mediated AmpC resistance genes

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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-lactamase(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 ceftazidime to detect inducible AmpC (current laboratory practice). In addition, a novel synergy-based method using cefotaxime +/– boronic 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.

Standard detection method	Boronic acid synergy-based method			
	AmpC +	Inducible AmpC	Neg	Total
AmpC +	7	1	3	11
ESBL and AmpC +	4	0	2	6
Inducible AmpC	0	2	3	5
ESBL only	7	0	7	14
Neg	0	1	0	1

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.

P2065 Detection of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking chromosomal ampC genes using the Rosco double disc synergy test and the E-test

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Objectives: to evaluate 2 commercial tests namely the Rosco double disc synergy test (Rosco Diagnostica, Taastrup, Denmark) and the Etest (AB bioMerieux, Solna, Sweden) to detect plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking chromosomal ampC genes.
Methods: A total of 48 isolates of Enterobacteriaceae (41 *Klebsiella pneumoniae*, 4 *Klebsiella oxytoca*, 2 *Salmonella* Newport, 1 *Proteus mirabilis*) were studied. They comprised 12 isolates with plasmid-mediated AmpC β-lactamases of the CIT-, DHA-, and FOX-type, and 12 isolates producing extended-spectrum β-lactamases (ESBLs) of the CTX-M and SHV-type. Except for ESBL-producers only cefoxitin-resistant isolates were included in the study. All isolates were tested for AmpC β-lactamase production with the following tests: 1) the Rosco double disc synergy test which is based on the synergy between ceftazidime or cefoxitin and cloxacillin, and ceftazidime+clavulanic acid or cefotaxime+clavulanic acid and boronic acid, 2) a new Etest for AmpC detection containing cefotetan/cefotetan + cloxacillin (a MIC ratio of $\geqslant 8$ or a deformation of the ellipse was interpreted as positive, a MIC ratio of <8 or a cefotetan MIC of $\leqslant 0.5$ mg/l was interpreted as negative).
Results: The Rosco synergy test detected 11 of 12 AmpC producers. In these isolates, at least 3 combinations of antibiotics showed a synergy phenomenon. One isolate harbouring an ampC gene produced double inhibition zones that were not interpretable and was thus omitted from the statistical evaluation. Of 36 isolates without AmpC, 34 (94%) showed no synergy phenomenon with any combination of antibiotics, while 2 showed a synergy phenomenon with only one combination. When interpreting only 2 or more positive synergy tests as indicative of an AmpC β-lactamase, sensitivity and specificity of the test was 100%. The Etest detected 10 of 12 AmpC producers. The MIC ratios of 3 isolates (2 isolates harbouring ampC and one without ampC) were not interpretable and thus omitted from the statistical evaluation. One AmpC-negative isolate gave a false positive result with a MIC ratio of >64. Sensitivity and specificity of the Etest were 100% and 97%, respectively.
Conclusions: Both the Rosco double disc synergy test and the Etest proved to be useful tools to detect plasmid-mediated AmpC β-lactamases

P2066 Routine susceptibility testing methods may fail to detect plasmid-mediated AmpC enzymes in Enterobacteriaceae

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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, ceftriaxone and ceftazidime by disc diffusion following CLSI guidelines and microbroth dilution (Microscan, Siemens Diagnostics, USA). Automated susceptibility testing to ceftazidime and ceftriaxone was performed using the N019 card, on the Vitek 2 Compact (bioMérieux, France) running system software version 02.01n. 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: CIT-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 disc 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 ceftriaxone 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 ceftriaxone, 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.

P2067 Cefditoren versus ceftazidime in inducer-substrate combinations for the evaluation of AmpC production in a disk approximation test

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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 morganii*, 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 $\geqslant$2 mm. Comparisons of percentages of AmpC isolates detected with the different substrates were performed with the Fisher's exact test, and reductions in diameters were compared by the Student's t-test. A $p < 0.05$ was considered significant.
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.

	CDN 10 μg			CAZ 30 μg		
	n	No. (%) of strains with reduction	Reduction (mm)	n	No. (%) of strains with reduction	Reduction (mm)
C. freundii	7	6 (85.7)	5.16±2.37	10	7 (70.0)	4.94±2.98
M. morganii	13	10 (76.9)	5.32±1.11[a]	13	11 (84.6)	3.92±1.59
P. rettgeri	7	3 (42.9)	3.47±0.25[a]	7	2 (28.6)	2.64±0.02
S. marcescens	11	11 (100.0)[b]	3.50±0.93	11	6 (54.5)	2.78±0.95
E. cloacae	22	16 (72.8)[b]	4.19±1.00	23	5 (21.7)	3.88±2.39
E. aerogenes	3	2 (66.6)	3.45±0.97	4	2 (50.0)	2.76±0.03
Total	63	48 (76.2)[b]	4.17±1.41	68	33 (48.5)	3.79±2.02

[a] Significantly ($p < 0.05$) higher reduction than CAZ.
[b] Significantly ($p < 0.05$) higher percentage of AmpC strains detected with CDN vs. CAZ.

P2068 Comparison of phenotypic techniques for the detection of metallo-β-lactamases in *Pseudomonas aeruginosa* from respiratory isolates

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Objective: The aim of this study was to compare different phenotype techniques for metallo-β-lactamases (MBLs) production in *P. aeruginosa*.
Methods: from January 2004 to December 2005, 86 consecutive, non duplicate carbapenems-resistance *Pseudomonas aeruginosa* isolates were collected from patients attending the National Institute of Respiratory Diseases in Mexico City. All isolates were processed to carbapenems susceptibility (imipenem and meropenem) by standard broth microdilution following CLSI guidelines. The EDTA disk synergy test (EDS), synergy test with double disc of imipenem (DD), EDTA-imipenem microbiological assay (EIM) and E-test was carried out by the screening of MBLs production. The presence of genes encoding blaVIM-like, blaIMP-like, or blaSPM-1 was confirmed by PCR primers and DNA sequence analysis.
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.

Test	Result	No. of specimens		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive	Negative				
EDS assay	Positive	5	33	100	59	13	100
	Negative	0	48				
DD assay	Positive	5	63	100	22	7.3	100
	Negative	0	18				
EIM assay	Positive	3	0	60	100	100	97.5
	Negative	2	81				
E-test	Positive	5	0	100	100	100	100
	Negative	0	81				

P2069 Heteroresistance contributes to variable carbapenem susceptibility levels in VIM-1 producing *Klebsiella pneumoniae* strains belonging to the same clone: consequences for routine susceptibility testing

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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 (XbaI-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 carbapenem 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, $\geqslant$1–>8 mg/L by WIDER and 0.75–>32 mg/L by Etest. Ranges for meropenem and ertapenem with Etest were 0.38–>32 and 0.75–>32 mg/L, respectively. Only one isolate with high level imipenem resistance (MIC > 128 mg/L) did not express porins OmpK36. 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 mayor 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 diference). 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 carbapenem 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. Carbapenem MICs are not good markers of MBL production and reliable phenotypic methods are needed to confirm the presence of this mechanism.

P2070 **Rising carbapenem resistance in *Pseudomonas* and Enterobacteriaceae at a large district hospital in northwest England – how relevant is antimicrobial disc susceptibility testing?**

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Objectives: Since Jan 2008, use of fluroquinolones [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 MICs.
Methods: BSAC standard methodology [DST] was comapred with MICs by E-test [AB bioMerieux] for Ceftazidime, Piperacillin-tazobactam, Meropenem, Imipenem, Ciprofloxacin, Gentamicin. Also Metallo-βlactamase [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).7 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 studied.
Conclusion: Disc-susceptibility testing correlates well with MIC testing by Etest for Enterobacteriaceae and *Pseudomonas*.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 Ertepenem 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**

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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 events 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**

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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**

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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. The water 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 37ºC 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 sample. The numbers of legionellae detected exceeded 102 cfu/100 ml in 65.3% of the samples. Legionellae contamination of water distribution system was higher in examined hospitals comparing to health resorts (56.7% and 38.8% respectively).
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 used 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 eradicating *Legionella* spp. from water distribution systems in selected hospitals of Lublin region

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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 10^2–10^3 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 (>55ºC) and temporal increase in the temperature up to 70ºC 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.

P2075 Invasive pneumococcal disease after systematic use of 7-valent pneumococcal conjugated vaccine. Multi-centre study with seven hospitals in Madrid, Spain

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Objectives: 7-valent pneumococcal conjugated vaccine (PCV7) was licensed in 2001 in Spain, however, in the Automous Region of Madrid, it was not included in the paediatric vaccination schedule until 2006. The objetive of this study was to determine the predominant serotypes and antimicrobial susceptibility of *Streptococcus pneumoniae* causing pneumococcal invasive disease (IPD) in the 7-valent pneumoccal conjugate vaccine (PCV7) era.
Methods: Multicentre retrospective study involving patients with IPD in seven hospitals in Madrid in 2008, attending 1,400,000 inhabitants. Antimicrobial susceptibility was tested using E-test®, following the Clinical Laboratory Standars Institute criteria. All the strains were serotyped using the Pneumotest latex and Quellung reaction at the Regional Public Health Laboratory. Urinary antigen detection was performed by Binax Now®.
Results: A total of 79 episodes were observed, 52 in adults and 27 in children, 23(29%) of the individuals were younger than 4 years, and 17(21.5%) were older than 65 years. *S. pneumoniae* was isolated from specimens of blood (73), pleural fluid (2), ascitic fluid (1), and CSF (3, two of them were also isolated in blood). 13.9% of IPD cases were caused by serotypes included in PCV7, 9 adults and 2 children. One of these children, 4 months aged, with a primary bacteraemia caused by *S. pneumoniae* serotype 9V, had received two vaccine doses. The most prevalent serotype was 19A (22.8%), followed by 1 (17.7%). Neither of them were included in PCV7. The MIC90 for penicillin was 2 ug/ml (range; 0.016–4) and for cefotaxime 2 ug/ml (range: 0.016–4). The susceptibilities to levofloxacin, erythromycin and clindamycin were 96.2%, 81% and 86% respectively. One *S. pneumoniae* strain isolated from CSF had reduced susceptibility to penicillin (MIC: 0.75 ug/ml) and cefotaxime (MIC: 1 ug/ml). In 27 of the 52 adult patients *S. pneumoniae* urinary antigen was required. Antigen detection was positive in 18 cases (sensibility: 66.6%).
Conclusions: A worrying emergence of serotypes not contained in vaccine such us 1 or 19A is observed. This could in future decrease the effectiveness of the vaccine or lead to changes in the epidemiology of pneumococcal disease. We expect PCV13 to be available in the near future. 62% of the isolates in our study belong to one of the serotypes included in this new vaccine. Additional surveillance studies focused on the clinical and molecular epidemiology of IPD are needed to understand the impact of PCV7 and the upcoming PCV13.

P2076 Comparison of invasive pneumococcal disease rates in Alaska and northern Canada following PCV7 introduction

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Background: The International Circumpolar Surveillance Project is a population-based surveillance network for invasive bacterial disease among the following Arctic countries: Norway, Sweden, Finland, Iceland, Greenland, Canada and the US (Alaska). The 7-valent pneumococcal conjugate vaccine (PCV7) has been used for routine infant immunisation in Norway since 2006, Alaska since 2001, and in northern Canada since 2003; however, the start date varied by region in the latter.
Methods: Invasive pneumococcal disease (IPD) data from the North American Arctic (1999–2007, Alaska and Northern Canada, total population 783,500) were analyzed to determine predominant clinical

findings, disease rates, serotype distribution and antimicrobial susceptibility.
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.

P2077 Usefulness of C-reactive protein and procalcitonin determination for the differential diagnosis and guidance of the clinical management in pneumococcal and *Legionella* pneumonia

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Objectives: Community acquired pneumonia is a frequent and potentially life threatening infection, Patients' outcome greatly depends on the timely initiation of adequate empirical therapy which is challenging in patients suffering from pneumonia caused either by *Streptococcus pneumoniae* or *Legionella pneumophila* since these pathogens have different antibiotic susceptibility patterns. We thus investigated the potential of widely used tests for serum markers of inflammation to differentiate between those pathogens and to guide the clinical management of these infections.
Methods: We retrospectively analysed the records of 60 patients with severe *S. pneumoniae* (37) or *L. pneumophila* (23) pneumonia. Diagnosis was confirmed by urinary antigen tests, blood culture and/or serology. The results of C-reactive protein (CRP), procalcitonin (PCT), blood counts, liver and kidney function tests were available on day 1 and day 5 of admission.
Results: While we found no significant differences between patients suffering from *S. pneumoniae* and *L. pneumophila* infection at admission in terms of CRP levels, leucocyte counts, haemoglobin concentration or creatinine levels, PCT ($p < 0.03$), serum sodium ($p < 0.02$) and chloride levels ($p = 0.005$) were signficantly lower, and ferritin levels significantly higher ($p < 0.01$) in pneumonia patients suffering from *L. pneumophila* than from *S. pneumoniae* infection. Moreover, patients with *L. pneumophila* pneumonia more frequently suffered from an underlying chronic obstructive pulmonary disease ($p < 0.02$), and were more frequently admitted to the intensive care unit ($p < 0.05$). In both groups, increased PCT levels on days 1 and 5 and high CRP concentrations on day 5 predicted a poor clinical outcome concerning the need of mechanical ventilation or death.
Conclusions: PCT may harbour diagnostic benefits in the early differential diagnosis between *S. pneumoniae* and *L. pneumophila* pneumonia. In addition, high PCT levels at admission and follow up are associated with an increased risk for an adverse clinical course and may prompt physicians to consider early intensive care admission.

P2078 Genetic polymorphisms of innate immunity and susceptibility to pneumococcal infection

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Objective: To investigate whether diverse genetic variants of innate immunity (MBL, TLR2, TLR4, and FcgRIIa) that cause hyporesponsiveness 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/mm^3) 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 ($p < 0.001$). In addiction, TLR2 Arg753Gln polymorphism was present in 5.6% of patients with pneumococcal disease and in 0% of control subjects ($p = 0.156$). 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

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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%, 9% 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%, 15% and 13% 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.

P2080 Unexpectedly high prevalence of *Chlamydophila psittaci* in patients with CAP

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Background: Community-acquired pneumonia (CAP) is caused by many different pathogens; most identified micro-organisms are *Streptococcus pneumoniae* and *Haemophilus influenzae*. Previous studies have shown that in approximately one third of patients with CAP no causative agent is found. As part of a prospective trial to evaluate the additional value of corticosteroids in the treatment of CAP, an extensive diagnostic algorithm for the identification of causative agents in CAP is currently used in our hospital. Here, we report on the relative contribution of respiratory pathogens identified in this study.
Material and Methods: Pathogen identification was performed by culture of sputum and blood. Urine samples were taken for antigen testing of *Legionella pneumophila* serogroup 1 and *S. pneumoniae* cell wall antigen. Polymerase chain reactions (PCRs) were performed on sputum to detect *Legionella* species, *Mycoplasma pneumoniae*, *Chlamydophila psittaci* and *Chlamydophila pneumoniae*. Serological testing was performed for antibodies against *M. pneumoniae*, *Coxiella burnettii*, *C. psittaci* or respiratory viruses (influenza A and B, parainfluenza viruses 1, 2 and 3, adenovirus and respiratory syncytial virus). IgG and IgM antibodies to *Legionella* were determined on day 1 and 30 by ELISA. For viral cultures, pharyngeal and nasal samples were taken.
Results: So far, 70 patients with CAP have been included in our study. *S. pneumoniae* (n = 19; 27%) and *H. influenzae* (n = 6; 9%) are the most frequently found causative agents of CAP. Remarkably, 5 of 70 (7%) patients had a *C. psittaci* pneumonia. All five patients demonstrated a four-fold rise in antibody titre; two patients had a positive PCR for *C. psittaci*. After diagnosing psittacosis, all five patients were – again – asked for bird contacts. Two patients remember contact with dead birds (a blackbird and a budgie) in the week before admission. The other three patients did not recall any bird contact. One patient needed ICU admission because of respiratory insufficiency, but responded well to erytromycin. Three patients recovered before serology testing became positive and one patient recovered after antibiotic switch to claritromycin.
Conclusion: *C. psittaci* has a remarkably high prevalence in our ongoing study in patients with CAP. *C. psittaci* may result in severe pneumonia and requires specific treatment. Bird contact known to the patient is not a conditio-sine-qua-non for a *C. psittaci* pneumonia.

P2081 Prevalence of *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila* among patients with atypical pneumonia in central Greece

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Objective: To evaluate the prevalence of the most common atypical pulmonary pathogens, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila*, among patients with community acquired pneumonia (CAP) in Central Greece.
Methods: One hundred and forty four patients (104 males and 40 females) mean age 67 years (17–92), hospitalised during 2008 in the University Hospital of Larissa with CAP, were included. On the admission, sputum, urine and serum from each patient were collected and sent to the Microbiological Laboratory for further analysis All sera were tested for the presence of antibodies against the above described microorganisms by an enzyme-linked immunosorbent assay (Alphadia). DNA extracted directly from the purulent portion of the sputa was used for PCR with primers targeting genes specific for *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila*. Urine samples were tested for the presence of *L. pneumophila* antigen (Binax).
Results: Among 144 serum samples, 17 (11.8%) were positive for antibody titres of acute infection against atypical pulmonary pathogens: 13 (9%) of them against *M. pneumoniae* (11 IgA, one IgM and one IgM and IgA), two (1.4%) were positive for IgM and IgA against *C. pneumoniae*, one (0.7%) was positive for IgM against *L. pneumophila*, while one more (0.7%) patient had serological data of dual acute infection (IgM and IgA against *M. pneumoniae* and IgM against *C. pneumoniae*). Sputum PCR results showed that, only one out 17 samples with positive serological tests carried DNA of *L. pneumophila*; the urine test of this patient was also positive for the detection of *L. pneumophila* antigen. On the other hand, two out 127 patients with negative serological profiles for atypical pulmonary pathogens, gave positive PCR results for *M. pneumoniae*. Repetition of *M. pneumoniae* antibodies two weeks latter showed that these two patients remained negative.
Conclusions: In Central Greece, during 2008, the prevalence of atypical pulmonary pathogens in adult population was 13.2%. Combination of serological and molecular tests is recommended for the accurate diagnosis of atypical pneumonia.

P2082 Community-acquired pneumonia in elderly patients with and without type 2 diabetes mellitus

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Introduction: It remains uncertain whether community acquired pneumonia (CAP) is more severe and carries worse outcome in diabetic patients. The aim of the present study was to investigate and to compare clinical features and outcome in diabetic and non-diabetic elderly patients with CAP.
Materials and Methods: All records of patients aged $\geqslant$65 years, with and without type 2 diabetes mellitus (DM) and CAP, cared for at the department of Medicine of the University Hospital of Heraklion from January 2004 to December 2007 were retrospectively reviewed. The severity of CAP was assessed using the CURB65 score.
Results: Eighty patients with type 2 DM and CAP (33 females; 41%), with median age 77 (range:65–91) years, and 95 non-diabetics suffering of CAP, age- and sex-matched serving as controls, were evaluated. Fever upon presentation was more common among diabetics than controls (88.8% vs 72.6%; $p < 0.01$). One hundred eight out of 175 patients (62%) had a single lung opacification, while 67 (38%) presented with multilobar or multi-segmental opacifications. No differences in opacification types were observed between the two groups. Diabetics had significantly lower CURB65 score on admission (2.08 ± 0.84 vs 2.37 ± 0.95; $p < 0.05$). No difference in the median time to defervencence was observed between the two groups [3 (1–8) vs 3 (1–6) days]. However, DM patients had

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

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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 tubersulosis.

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 10(7)/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.